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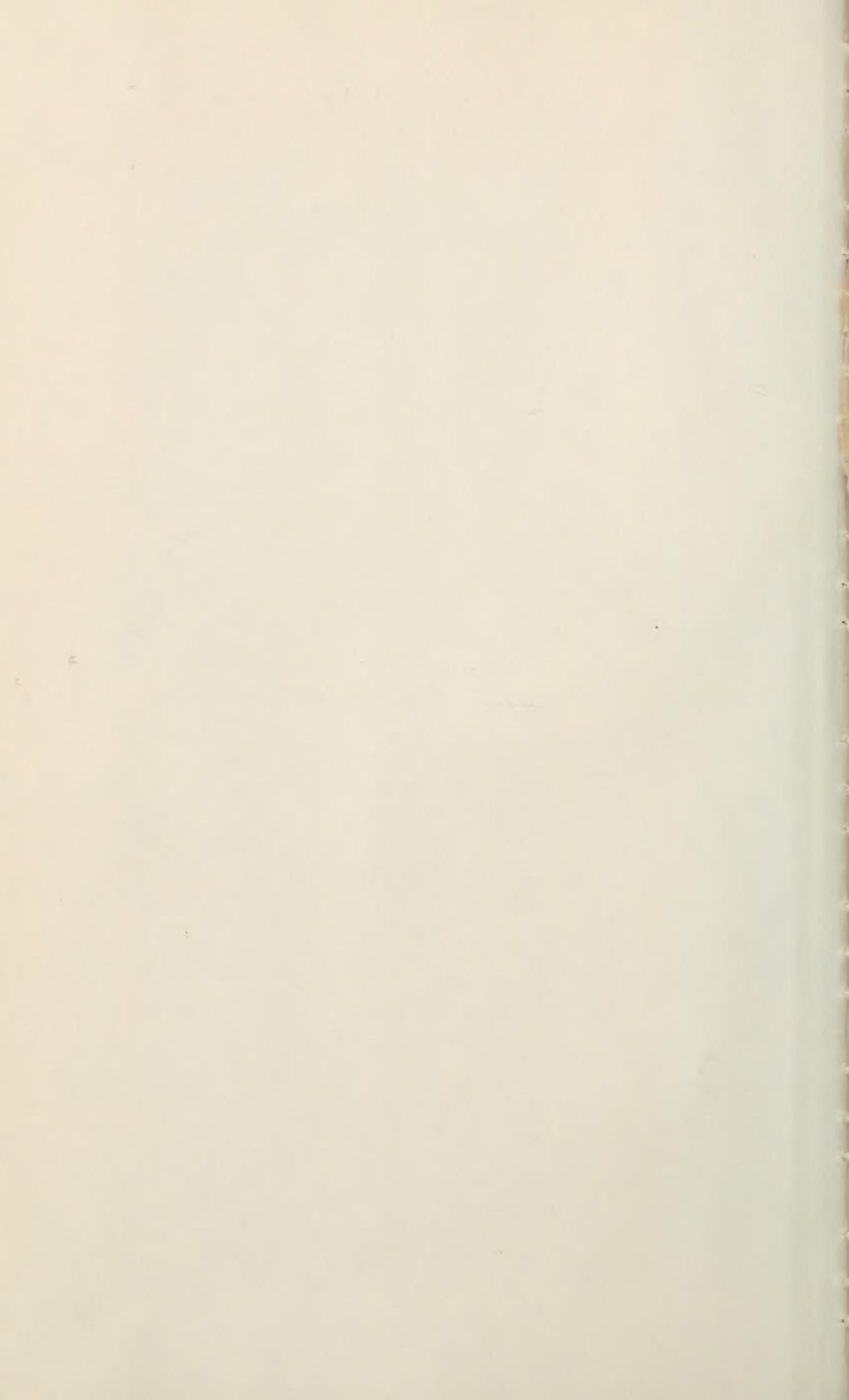
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GENERAL PHYSIOLOGY

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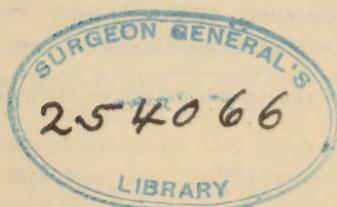
A TEXT BOOK

OF

GENERAL PHYSIOLOGY
FOR COLLEGES

BY

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FIRST EDITION



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PREFACE

This book is designed for use by college students who have studied introductory biology, chemistry and physics but have not studied the organic and physical chemistry that seem prerequisite to the intelligent use of the advanced text books of physiology and biochemistry which are now available. The material formerly taught in short courses entitled Elementary Physiology is now largely included, in many college curricula, in courses in General Biology. There seems to be a demand among students who have had general biology for a college course in physiology. The text books available for them are either too difficult or else contain little that is not already familiar.

General physiology has recently come into a new era. Research work of the experimental biologist, whether he started as a student of human physiology, of plant physiology, of comparative anatomy or of cytology, has alone put a new face on modern physiological science. When one also considers how the development of physical chemistry has entirely reshaped conceptions of the architecture and the behavior of living matter while even the findings of the new atomic physics have already reached their application in the field of biochemistry, the time seems ripe to cast the teaching of physiology in a new mold. There are already excellent texts written in the light of the newer knowledge. They are designed, however, for the advanced student. There is still little tendency to adapt the teaching of physiology in elementary form to the modern point of view. Although physical and organic chemistry are inextricably interwoven with physiology their study too seldom preceeds that of physiology. This work is designed, therefore, for those who want to study physiology in its essentials but who lack extensive preparation.

I have endeavored to describe physiological phenomena as energy transformations and to write, whenever possible, from the point of view of the law of mass action as it applies to dynamic equilibrium. Although I realize that in the present state of physiological research and of methods of teaching elementary science, this point of view does not afford the student as easily comprehended a picture as do others, yet I feel that it is the stand of the present-day leaders in physiological research and is the only ground from which the student can truly see the rapid modern development of knowledge of the processes of life.

I have aimed to lay emphasis upon the fundamental facts of physiology. On this account the special aspects of the subject are described, for the most part, only as illustrations of general principles. This

entails the omission or abbreviation of some of the facts of special physiology. Muscular contraction, external respiration, the function of receptor organs and the chemical composition of tissues are more limited in application to general physiology than are some other subjects and are therefore treated with comparative brevity. Reproduction, a subject requiring extended treatment in order to be adequate and one which has limited applications to general physiology, has been entirely omitted to give space for treatment of other subjects.

The text-book use of the names of investigators is apt to be confusing to the student who has not specialized in the subject to the extent of reading reports of its original researches. Nevertheless a text book should open avenues of such reading. It seems advisable, then, to refer to original workers in some cases and yet to avoid overloading the text with such references. The choice of those to be mentioned must be somewhat arbitrary. The principles which guided the choice were: To mention those whose work has been of a pioneer character or has led to the formulation or modification of fundamental physiological theories and to call attention to the names of investigators whose work seemed particularly useful for reading in the original. I beg the indulgence of others whose work has been reviewed without such acknowledgements.

No attempt at complete bibliographies has been made. Lack of space would alone preclude this. This omission is not serious because for nearly every aspect of the subject extended bibliographies already exist and are no more difficult of access than are the originals. Short lists of references to monographs, review journals and treatises which contain bibliographies are given at the ends of chapters.

To the publishers and authors who have kindly allowed the use of material for illustrations, my hearty thanks are due. Individual acknowledgements will be found with the legends of the several figures. My appreciation of the helpfulness of those who have kindly given criticism of the manuscript is very real. I wish especially to acknowledge my indebtedness to Dr. A. D. Mead and Dr. J. W. Wilson of Brown University, for useful suggestions.

P. H. M.

PROVIDENCE, R. I.
August, 1923.

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INTRODUCTION

A man and a child are looking at a plant. They notice that most of its leaves are green and are sturdily held in position. They speak of these leaves as alive. They notice that one leaf is faded, drooping, sere and crumpled. They conclude that it is dead. The child asks what killed it. The man replies as best he can; but in his own mind he asks more searching questions.

"What ever made it alive? What do we mean by saying that one structure is living and another dead? Of what stuff is it composed? Why must it have sunshine, moisture and warmth in order to live? What foods did it use and how did it use them? How did it grow? Of what use was the leaf to the plant as a whole? Could it have lived and grown except as a part of the entire plant organism?"

Human efforts to answer these and similar questions about living things have developed the science of physiology.

The Scope of Physiology.--Physiology is *the study of living things in action*. It deals with all the processes, physical, chemical and physico-chemical, that go on in what we call living matter while it is typically alive and while going through the stages that lead to death. All processes involve transformations or transfers of energy. They are dynamic. Physiology, dealing as it does with the processes of life, is sometimes called *the dynamics of living matter*.

Recognized as having that breadth of scope, it is seen to be more than the study of the healthy functioning of the organs of the human body, even though the emphasis, very naturally placed on this type of study, might lead one to suppose that human physiology was the entire subject. This part of physiology is fundamental for hygiene, medicine and surgery and for many aspects of sanitation. It therefore stands out as a practical and important part of physiology. But physiology is more than this. It is even more than the study of the life processes in all the plants, animals and microorganisms that are directly related to agriculture, to disease prevention and to other practical human enterprises; for physiology in its broad or general sense deals with *all living things*.

Even if the scope of physiology were arbitrarily restricted to studies that have an obvious and immediate practical application, it would soon break away from such unnatural restrictions; partly because scientific

curiosity, which is a powerful motive in research, is like all other types of intellectual curiosity in that it knows no bounds. Moreover the seemingly least practical researches often lead to the most far-reaching discoveries which, just because they are fundamental, eventually come to have especially wide practical application. This statement would seem to be self-evident in these days of practical use of electricity which, even at the time of Faraday's epoch-making researches, seemed to have only theoretical interest. But even now some students of physiology ask why they should study the chemistry of proteins since the subject seems at first sight to be a mere matter of scientific interest. Fortunately this feeling did not shut off investigations of protein chemistry which laid one of the corner stones of the modern science of human nutrition. Even so practical a problem as the cure and prevention of the dread disease, pellagra, has been shown to depend for its rational solution upon the chemistry of the proteins.

Other instances might well be brought forward to show the interdependence of general physiology and human physiology but the above will suffice to indicate the value of a broad gauge view of the scope of physiology.

"Its subject matter," said Huxley, speaking of physiology, "includes a large moiety of the universe."

Physiology, in dealing with the activities of all forms of life, seeks to discover the nature of the life process. What chemical reactions occur in living matter? How is the character or the rate of these reactions influenced by the presence of food, by temperature changes and by all other environmental conditions? What are the mechanisms by which motion, growth and other physical functions of living things are made possible? What determines the intricate form patterns which exist throughout the material that we call living? How does this intricate organization of material determine, in its turn, the behavior of a living thing? These questions are representative of the important problems of physiology. The physiologist stands face to face with the deep mystery of the nature of life.

The Methods of Physiology.—The beginner in physiological studies very soon comes to realize how wide a variety of scientific methods is used in his work. At one time he may be studying the movements of protoplasm in an amoeba by the use of methods that he had previously heard about only in the laboratory of physics. At another time, he may be studying the oxidation of sugar in the human muscle employing the technique and the theories learned in chemical laboratories. At still another time, he may consider how the salts in farm soil influence the acidity or alkalinity of the soil water and thereby affect the growth of crops. For this work he requires a training in physical chemistry. These are but illustrations of the general fact that physics, chemistry

and physical chemistry are applied to physiological studies of microorganisms, animals and plants.

Quantitative precision is an essential characteristic of physiological methods. Excepting in so far as they are quantitative, investigations do not greatly advance an understanding of the living mechanism. The need of exact measurement is apparent in all science. The laws of motion and all other fundamental generalization of physical science are established only in so far as the precision of quantitative method permits. Newton's law of gravitation, for example, though based upon comparatively correct measurements, appears to be an approximation which requires to be supplemented in accordance with Einstein's more correct law of relativity, based upon greater refinement of measurements. Similarly, physiology is founded upon experimental methods which are able to further knowledge of life only as they continue to approach exactness. This is more strikingly true of physiology than of certain other biological sciences because physiology deals with processes, with continual changes, and not with static conditions. The detection of minute changes, such as some of those that occur in living matter, demands the highest degree of accuracy. On this account, physiology has depended for its development upon the improvement in exactness in the better instruments and methods of physics and chemistry. Without the sensitive galvanometer, the refined spectroscope, the delicate chemical balance and scores of other instruments of precision, physiology would be at a serious loss. Lacking the modern electrometric methods for determining acidity, the newer methods of photography, especially by X-rays, the delicate methods for estimating amounts of sugar and very many other refined methods of physics and chemistry, physiological progress would be almost at a standstill. Physiologists have not always followed the lead of pure physicists and chemists, but have invented instruments and methods of precision as the need for them arose in the course of physiological investigation; but on the whole, physiological advance has been conditioned by the improvement of methods in the so-called physical sciences.

The Relation of Physiology to Other Biological Sciences.—Physiology is so closely associated with her sister sciences in the family group of Biology that she is powerless to advance her work without the constant cooperation of the others. This dependence is a little less conspicuous in the case of some of the other members of the family. Anatomy, for example, can carry on without reference to other studies; although the study of form and structure can not be complete or satisfying without some reference to the adaptation of each structure to the working processes which constitute its physiology. Much of physiology, however, is obviously unintelligible without reference to structure. So close is the relationship of the biological sciences to one another that a picture showing them as the branches of one tree is perhaps better than that repre-

senting them as a family of individuals. Physiology may be regarded as one of the unifying principles running through all parts of the tree as the sap circulates through root and branch.

The following grouping of the branches of biology attempts to indicate a few of the ways in which physiology permeates these branches and is itself dependent upon them for its very existence. This grouping should make clear the fact that all biology is one. The naming of the different branches as though they were distinct is merely a matter of convenience and arises from the circumstance that one worker in the span of a lifetime is usually able to prune and pick upon only one branch.

		Physiology (chemical and physical processes)
		Anatomy (gross structure)
		Histology (microscopic structure)
	Morphology (structure)	Embryology (development of structure, studied partly by physiological method)
of animals	ZOOLOGY	Cytology (morphology and physiology of cells)
		Pathology (morbid morphology and physiology)
		Psychology (mental phenomena, studied largely by physiological method)
		Ecology (adaptation and other relationships of organism to its environment, studied chiefly by physiological method)
		Taxonomy (classification, based chiefly on comparative anatomy but partly on physiology)
BIOLOGY, the science of life	of plants BOTANY	Physiology
		Morphology
		Cytology
		Pathology
		Ecology
		Taxonomy
Grouping into plants, animals, etc. is based chiefly on physiology		Each of these has its physiological aspects as in corresponding zoological studies.
	of those microorganisms and other forms, difficult to classify as true plants or true animals,	
	BACTERIOLOGY, dealing with plant-like microscopic forms,	Each of these branches of biology subdivides into physiology, morphology, cytology, pathology, ecology and taxonomy, and each of these subdivisions has its physiological aspects.
	MYCOLOGY, dealing with fungi,	
	PROTOZOOLOGY, dealing with animal-like microscopic forms	

This scheme does not pretend to be a complete classification of the biological sciences. It does not show all of the diverse groupings of parts of these arbitrary subdivisions as they are conveniently put together to constitute certain studies, such as evolution, genetics, eugenics, agricultural biology, forestry, paleobiology and a long list of others. A complete representation of all such relationships would be a very large chart. It would be of only temporary value because new centers of biological interest are continually arising, each drawing around itself a new grouping of biological studies.

The relationship of physiology to sciences other than the biological ones is also very close. Chemistry, physics and physical chemistry are the very foundations of physiology. Mathematics is the logical language for its expression because physiology deals with active processes, dynamics, which can be satisfactorily understood only by quantitative measurements. Sociology and economics join forces, at times, with physiology.

Huxley said: Its position is midway between the physico-chemical and the social sciences. Its value as a branch of discipline is partly that which it has in common with all sciences—the training and strengthening of the common sense; partly that which is peculiar to itself—the great exercise which it affords to the faculties of observation and comparison; and I may add, the exactness of knowledge which it requires on the part of those among its votaries who desire to extend its boundaries.

It may possibly help to clarify the idea of the nature of physiology to give a specific illustration of a boundary between it and one of its sister sciences, psychology, for instance. Suppose one is investigating memory. So long as the method is that of introspection, a process of pure logic applied to human memory, one is working in the field of pure psychology. But when investigating the nature of the chemical reactions that occur in the brain as an accompaniment to the memory process, one is working in the border land between psychology and physiology and is using the physiological method. The latter type of investigation has been carried on by men who are called psychologists and also by those who are called physiologists.

Any aspect of biology dealing with chemical, physical or physico-chemical processes in living things is physiology.

The aims of physiology transcend its scope. It aims higher even than those splendid aspirations to further the progress of medicine, surgery, sanitation and agriculture. Physiology has for its final goal, far away and dim as yet, but steadily held in view, nothing less than a complete interpretation of life phenomena in terms applicable to non-living mechanisms—in short, a physico-chemical explanation of life.

The scope, the methods and the aims of physiology serve to define it as: *The physics and chemistry, the dynamics of life.*

The Fundamental Laws of Biophysics and Biochemistry—The final goal which physiology has set for itself reveals the faith which animates its workers. The golden-text of this faith is: All vital phenomena may be explained by the laws of physico-chemical science. That physiology cannot make those explanations now need not preclude hope for the future. Physiologists assume, at least, that the fundamental laws of biophysics and biochemistry are those of ordinary physics and chemistry. This means: (1) That life is regarded as *no more—and no less—than a very complex series of energy transformations*; and (2) that the law of the *conservation of energy* is assumed to apply to all those phenomena that we call living just as it does to all others. When foods are utilized by the processes of oxidation in plants and animals, the energy so liberated, though appearing in diverse forms, may all be measured and its total amount foretold from the fuel value of the food. Conversely, any event in living matter can only be a transformation of energy coming from some detectable source. In short, living matter does not produce something out of nothing.

To say that the physiologist *assumes* these laws to hold true is not to signify that he attained to that faith, “lightly or inadvisedly.” Such faith is, to be sure, daring; because the phenomena of human life, indeed, even those of the simplest forms of life, appear to be very different from other phenomena. The assumption is the outgrowth, however, of intensive research, covering more than a century. A few illustrations, taken from the history of physiology, will serve to emphasize this statement.

One instance is found in the history of knowledge about respiration. Opinions concerning this process were formulated, scientifically, up to the middle of the eighteenth century, under puzzling difficulties. Writers tried to reconcile the fact that air was equally necessary for life and for fire with the then current theory that “vital spirits,” taken from the air by breathing, were necessary to maintain the heart beat. The dilemma was very real then, although the explanation seems so clear now. It did not become clear until Lavoisier worked out, during the years 1771–1780, the chemical composition of the air and showed that “vital spirits” consisted of nothing more than oxygen. It is equally necessary for life and for fire. As Lavoisier and a long line of other researchers have shown, oxidation, whether it occurs in a living organism or in the flame of a tallow candle, may begin with the same materials, yield the same final products and utilize oxygen for the process in entirely comparable, though not identical ways.

Another instance is seen in the story of researches upon the energy balance in living animals—attempts to show whether or not the living mechanism obeys the law of the conservation of energy. That the body evolves no energy save that potentially present in its food is not easily made apparent because animal energy is liberated in several forms and

in various ways. Some of it appears as heat and leaves the body through the skin, the lungs and the excretory organs. Some of the energy goes into the form of mechanical work and is expended in large numbers of different muscles. Other portions are transformed into potential energy to be stored in new growth of body tissues or in fat deposits or in other forms.

Referring to just one of these forms of animal energy, heat, it was said as late as 1849 after the discovery of the law of the conservation of energy:

The phenomena (of animal heat) are evidently so complex that it is scarcely probable that one will ever be able to submit them to calculation.

The calculation could not be made, indeed, until chemistry and physics had developed methods of accurate measurement. The animal calorimeter (Chap. XXIV) met the need. With its aid Rubner was able to show in 1894 that the total amount of heat evolved in the body of a resting dog is equal, within the limits of experimental error, to the quantity that should be expected from the amount of bodily oxidation occurring simultaneously. The amount of oxidation could be reckoned from the quantities of various nutriments and the amount of oxygen utilized by the animal. Experiments similar to Rubner's were made, a few years later, by Atwater and Benedict. They used a still more elaborate apparatus with which they were able to show that the muscular work of a man is just equivalent, in terms of energy, to the difference between the total energy evolved, measured by the total oxidation, and the energy expended in his body as heat and other forms of energy. Results, as obtained by Rubner and subsequently by Atwater and Benedict, have been confirmed and extended by other investigators; so that there is convincing proof of the theory that animal life proceeds in accordance with the law of the conservation of energy.

One more illustration is taken from recent physiological progress. The mechanistic nature of even that vital event which starts the development of an ovum has been demonstrated. For this process, fertilization by the living sperm cell was formerly supposed to be necessary in the case of all sexually reproducing organisms. Yet such is not the case. Certain non-living agencies are equally effective. Eggs have been made to develop by exposing them to certain salt solutions, to dilute acids or to suitable pressure. Eggs of numerous species of animals have been thus stimulated successfully. When ova develop in response to such stimuli the process is called artificial parthenogenesis. The experiments usually terminate with the death of the resulting organism at an early larval stage; but with certain kinds of technique, growth to the full adult stage has been observed. Loeb has effected artificial parthenogenesis of frog eggs by pricking each one with a needle. Although a large proportion of the eggs develop only partially before death, yet from among them, adult fatherless frogs have been raised in sufficient numbers to demonstrate

that the stimulus for instigation of complete development need be nothing more than a purely mechanical force.

The three above instances, given as demonstrations of the physico-chemical character of vital processes, are merely representative of many similar illustrations that might be drawn. Serious objections to a physico-chemical interpretation of life in general or of certain special vital processes have frequently been raised; but, one by one, many of them have been answered by researches that were as significant as these representative ones. Although every objection is not yet answered the progress already made has been sufficient to encourage further work in the faith of an ultimate solution.

The Physiologist's Philosophy.—The tendency of physiology to explain animal behavior and even human behavior, in terms of biophysics and biochemistry, has received much adverse criticism. Inasmuch as physiological explanations of the reactions of the nervous system seem to place even human volition in the category of mechanical reactions, the complaint against physiology reflects a feeling of revolt in the minds of many critics. They cannot accept the philosophical implications of the physiology of the brain because the conclusions seem to do away with our traditional conceptions of mind and personality. Judgment, character, freedom of the will, are reduced, physiologically, to mere results of physico-chemical processes which so affect nervous structures that a certain definite act is unavoidably caused by a given set of conditions.

Any one who feels this revolt should at least attempt to sympathize with the physiologist's point of view. It is admirably presented in Verworn's "General Physiology" in the sections on "The Relation of Psychology to Physiology" and on "Vitalism." After consideration of Verworn's statement of the idealistic philosophy which even the mechanistic physiologist cannot evade, the conception of a human being as a mechanism does not seem revolting. Philosophies have been built upon dualism. The older ones represented mind (spirit) and matter as the two primal elements. The newer ones take into account the modern science which has shown matter to be merely the regions in which are certain combinations of electrical energy. The modern dualistic contrast is therefore between energy and mind. But the scientist generally rejects the dualist philosophy and prefers to be a monist. Mind and energy are regarded as one and the same. The monistic scientist who is intellectually honest cannot escape the claims of idealistic philosophy so that he is forced to view mind as the one reality. He may call it by names other than mind. He may call it Spirit. He may call it the sum total of the energy of the universe. Perhaps it does not matter what he calls it. The concept may be the same in any case.

Nevertheless, the *scientific* explanation of physiological phenomena must be in terms of energy. But even a fundamental scientific explana-

tion could go no further, in the light of present knowledge, than to reduce all processes, even those of life, to terms of the movements of electrons and protons and the behavior of their attendant fields of force. Electrons and protons are names which we give to our *concepts* of the centers of unit charges of negative and positive electricity. Their fields of force are purely *imaginary conceptions*, postulated to aid in the discussion of electrical phenomena. But we know nothing at all about the origin and the ultimate nature of unit charges and fields of force. We hope for an ever larger and more unified knowledge about their reactions to constitute atoms, molecules and ions, to radiate energy, and to effect new arrangements of energy. But from what stuff might one attempt to produce an electron? How should one attempt even to state the characteristics of a field of force except in terms of our *concepts* of attraction and repulsion? If what we call the fields of force of electrons and protons are immortal, as the law of the conservation of energy undoubtedly implies, what was their primal origin? Do they possess that out of which consciousness grows? Such questions may seem out of place in a scientific discussion, because they belong in the realm of philosophy. They are stated here, however, because the lack of any *scientific* answer to them shows that the limitations of science are just as apparent in any effort to explain the nature of obviously mechanical phenomena as they are in attempts to explain life. A mechanistic conception of human behavior need not impair the dignity with which we clothe our ideas about the human spirit, but should rather raise to the same high plane our respect and even our devout appreciation of those phenomena which we call inanimate. However successful mechanistic physiological explanations may be, they do not explain the infinite out of life. They merely explain life all the more clearly in terms of the infinite. Clear thinking men have found science only deepens their sense of the spiritual dignity of human life by showing its relations to the universe as a whole.

The Arrangement of Subjects.—Granting that living matter is a mechanism, one may undertake the study of the living organism in a manner analogous to corresponding inquiries into man-built machinery. Suppose one undertook a systematic study of the automobile. He would doubtless first ask: "Has it an internal combustion motor; in short, to what fuels may it be adapted?" His next concern should be to take it apart and learn the structural details of the entire mechanism. He might then inquire into the materials chosen for the construction of all parts and master such problems as the explanation of the special usefulness of vanadium steel for certain structures and of ordinary steel for others. He should know the components that are required for a storage battery, the best materials for electrical wiring, for insulation, lubrication, etc. He would probably next undertake the assembling of the parts and would follow this work by a study of mechanics, including firing devices,

as well as piston thrusts, crank action and the like. He would also study the operation of controlling machinery, steering gear, brakes, switches and throttles. Another type of study would be that relating to the preparation, distribution, mixing and exploding of the fuel, including a study of the character and manner of ejection of waste gases. Another step would be to learn how various mechanisms are adjusted to each other in the completed machine: How cooling devices operate to regulate motor temperature, how the timer is adjusted to gas compression, and all other matters relating to the operation of the mechanism as a whole. By this time he ought to be ready to get into a car and learn to drive.

It is perhaps unnecessary to mention that the vast majority of automobile drivers have no more comprehensive knowledge of a motor car than has the average human being about the delicate mechanism that he is called upon to drive throughout life. The disadvantages that result from this state of affairs may be unavoidable, but this much, at least, is clear: The broader and more properly proportioned is one's knowledge of a machine the better is he prepared to operate it. Also when something goes wrong beyond his ability to repair, better understanding of his machine will enable him to discriminate between a competent mechanic and a mere "tinker." If there were more physiology there would be fewer fortunes made from the sale of quack remedies to a trusting but gullible public.

The idea outlined above will be followed in this book. Chapter I will show to what fuel the living mechanism is adapted. The procedure, corresponding to the next stage of motor study (examination of inner parts) is omitted because the study of anatomy and plant morphology so generally precedes that of physiology as to make such descriptions unnecessary except in special instances. Chapters II to VII will present descriptions of the foods and the composition of living matter—the stuff of which life is made. They will also be concerned with the specific uses of the several kinds of material. Chapters VIII and IX will deal with lubricants, the substances that permit the mechanism to run without destructive friction. Chapter X will present what little we know about reassembling of the mechanism. Chapters XI and XII will describe the firing mechanism and the mechanics of motion in the organism. Chapters XIII to XVI will deal with the controlling mechanisms. Chapters XVII to XXI will treat of the preparation, mixing and exploding of the fuel and Chapter XXII of the composition and elimination of the wastes. The later chapters will deal with those complex activities of animal bodies so closely interrelated to each other that they might well be termed the operation of the mechanism as a whole.

Like all analogies, the one we have been drawing may be carried too far. Sufficiently intelligent study of the automobile may enable one to become an automotive engineer and to direct the production of complete working

machines. Devoted and century-long efforts of some of the greatest minds known in the history of human knowledge have still left us quite unable to produce one drop of living matter.

Certain deviations from the general plan of the book will be noticed. They need no apology. The interrelationships of many phenomena necessitate the correlation of many facts and observations. This even entails some repetition; for no matter in what order subjects are discussed, each fact needs to be correlated to others some of which have not been previously presented. This is especially true of physiology for it calls in all branches of knowledge to a greater extent than do most studies. The arrangement of subjects, then, must be a compromise.

This unavoidable dilemma emphasizes the general principle that all knowledge is so interwoven that no isolated fact or group of facts can be intellectually satisfying. An observation attains greater clarity the more it is appreciated in relation to other phenomena. This is perhaps another way of saying that only infinite knowledge would be perfect knowledge. All knowledge, indeed, is a closely knit, intricate fabric. We catch glimpses of the warp and woof of its structure as we decipher, bit by bit, the scientific relationships of cause and effect. Although our best views can only encompass fragmentary bits of the whole, still the earnestly seeking soul finds its greatest joy in gaining an even broader, bigger, clearer view of the marvelous pattern of Nature's infinite weave.

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GENERAL PHYSIOLOGY

CHAPTER I

THE FOOD OF PLANTS AND ANIMALS

If we attempt to classify all living things as either plants or animals the task in some cases is not easy. Power of locomotion gives a distinctive contrast in many cases but not in all. Corals for example are included among animals but are fixed; while some plants even green ones, as for example certain diatoms, have considerable power of locomotion. Similarly the type of reproductive process shows no unfailing distinction between plants and animals, indeed, all criteria fail in some instances.

The contrast of most general application is in the character of the food requirements. Plants can utilize food of comparatively simple chemical constitution while animals must have more complex foods. For many, perhaps all green plants, nitrates, phosphates and sulphates of potassium, calcium, magnesium and iron together with carbon dioxide and water constitute adequate food for complete growth and reproduction. For plants that do not contain chlorophyll carbon is required in a form more complex than carbon dioxide—preferably in the form of sugar. A common mould, *Aspergillus*, grows abundantly on a solution containing the above salts and glucose.

Animals, on the other hand, must have more complex foods, more complex in the sense of being higher in molecular weight and more specialized in atomic grouping. The contrast between the complexity of food requirements of animals and those of plants is shown in the character of their nitrogenous foods. Among various representatives of the plant world, including bacteria and fungi, is found the ability to utilize nitrogen in the form of elemental nitrogen of the atmosphere, also in the form of ammonium compounds, nitrates, nitrites, amino-acids, acid amides, and many kinds of proteins; while an animal, aside from the restricted use of ammonium compounds, must obtain nitrogen as amino-acids or as proteins which it can convert, by the process of digestion, into amino-acids. Moreover, of the eighteen different amino-acids, known to be formed during the digestion of proteins by animals, at least five have been shown to be severally indispensable to the correct nutrition of mammals. Other substances, as will be apparent from later discussions,

are equally necessary as constituents of animal food; so that quite a varied array of foods must be available to an animal to nourish it correctly. If for any reason the diet is restricted to one or to a few varieties of foods, their composition must have a peculiar complexity to satisfy animal needs.

The Synthetic Power of Plants Contrasted with that of Animals.—Does this signify that animals have a distinctly more complex chemical composition than plants? There is no reasonable ground for such a conclusion. It is true that to casual observation animals appear to have a more complex structural organization than plants. One thinks of the marvelous complexity of the nervous system, for instance. But all the different classes of compounds which have been recognized as obtainable from animals have their representatives among the substances derived from plants. To be sure, every type of structure in both plants and animals, each kind of living tissue, has chemical individuality. No two have exactly the same chemical composition. But there is no evidence to show that complexity, in the sense of size of molecular aggregates, is any less in the simplest bacterium than in the most highly specialized cell.

It does signify, however, that plants have distinctly more *synthetic power* than animals. The materials which green plants take from their environment are not fuels as are the proteins, starches, sugars and fats of the animals food. But plants can synthesize their fuels while animals cannot. Animals must obtain part of their substance in ready made form, part of it in a highly prepared form. Only to a comparatively limited extent can they utilize materials in the stage of molecular simplicity which prevails among plant foods. The marvelous synthetic power which enables the plant to produce starch or cellulose from carbon dioxide and water or to form proteins from ammonium nitrate and other materials is one of nature's secrets which have been only partially revealed. This synthetic power is found in a more limited degree to be present in animals. They can synthesize the starch-like compound, glycogen, from sugar but not from carbon dioxide and water which plants use for synthesis of starch. Animals produce proteins from amino-acids but not from the ammonium compounds and nitrates which plants can thus use. The synthetic powers of plants and animals differ so widely from each other as to indicate fundamental differences in their characteristic chemical processes.

The Metabolic Cycle.—As a preliminary to a discussion of synthetic processes certain terms should be defined. *Metabolism* refers to all chemical processes occurring in living things or under their influence in their environment. It includes a great variety of chemical reactions. It is convenient to divide them into groups: The anabolic reactions, or *anabolism* and the catabolic reactions or *catabolism*. The former refers to the synthetic processes by which relatively simple substances of small molecular weight are built up into the more complex compounds

characteristic of protoplasm. Catabolism includes the breaking down processes by which substances of comparatively large molecular weight are split into the waste products of life. Anabolism is assimilation, catabolism is disintegration. It is a pronounced characteristic of living matter that both types of process can go on simultaneously in the protoplasm of the same minute cell. Thus while a plant leaf is building up carbon dioxide and water into sugar and starch it is simultaneously oxidizing these products to form carbon dioxide and water. Metabolic processes constitute a cycle, that ends often times just where it began, and always at such a point that some other organism, either plant or animal, may complete the cycle.

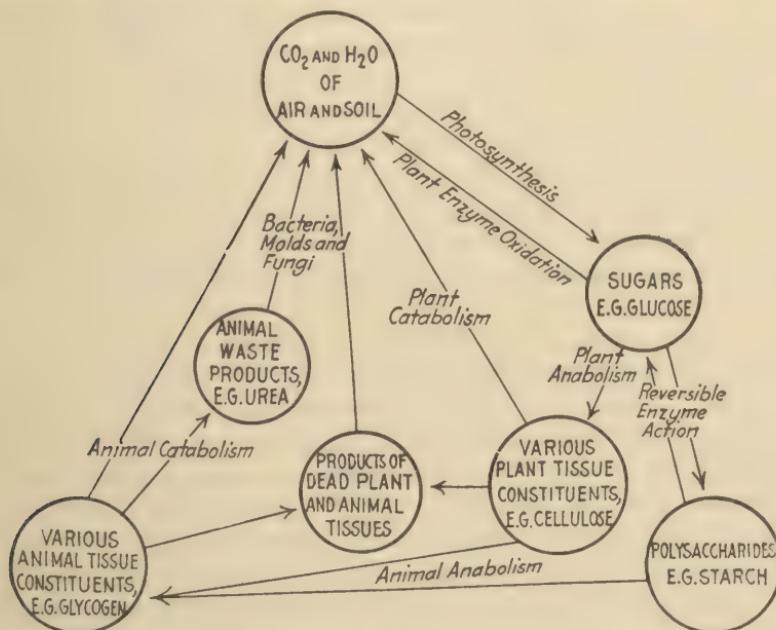


FIG 1 — Diagram to indicate some of the ways in which a carbon cycle can occur in nature.

The Carbon Cycle.—Starting with carbon dioxide and water, as taken from the air and soil by plants (See Fig. 1) photosynthesis produces some kind of sugar (glucose in the majority of plants). Plant enzymes may then change the sugar into a form capable of storage, some representative of the compounds called polysaccharides (starch the most familiar example). This may be restored to the form of sugar by enzyme action (See Chap. IX) and used when the plant seed is sprouting or when new leaves are forming. The sugar may be utilized as soon as formed or after its storage period but in any case will be oxidized eventually so as to produce CO₂ + H₂O. Some of the sugar is utilized in plant growth, synthesized along with other foods into tissue constituents (proteins, fats, cellulose, etc.). The stored polysaccharides or other constituents of

plant tissues may be eaten by animals and, after suitable preparation by means of the digestive process, built up into animal tissues. Animal life processes may then oxidize some of the material to carbon dioxide and water which are returned again to air and soil. Animals produce other waste products or, along with plants, go "the way of all grass" and reach the soil as dead organic matter; and, as bacteria, molds and fungi complete the cycle, the inevitable carbon dioxide and water result. This story is represented in schematic form in Fig. 1. Although it presents only a few of the possibilities of a so-called "carbon cycle" it serves to emphasize one fact: There is only one process by which carbon dioxide of the atmosphere can be made physiologically useful, namely, photosynthesis. Among the bacteria there are some minor exceptions to this rule, but in the main, carbon metabolism is dependent upon photosynthesis.

Photosynthesis.—All energy, manifested upon the earth, with the exception of that due to radioactivity and a few other minor sources, takes its origin from the sun. Life, a constant energy manifestation, is no exception to the rule. Of all living structures, however, only chloroplasts, the pigment containing granules of plants, can make a direct use of solar energy for synthesis of food. For this reason, photosynthesis is of fundamental significance in physiology and is, indeed, indispensable to human life.

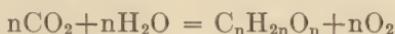
The green pigment, chlorophyll, is requisite for photosynthesis. This fact has been known for nearly one hundred and fifty years. Its proof may be furnished by various experiments; but the mere observation that photosynthesis does not occur in the yellow or colorless parts of a variegated leaf even while the green portions are carrying on the process, is proof enough. Chlorophyll occurs in very thin coatings upon the surfaces of chloroplasts. The latter are microscopic granules that are very numerous in the cells of the green parts of plants. Although chlorophyll is generally accompanied by other colored compounds, it alone is effective for photosynthesis in green plants. Chlorophyll can be readily separated from other leaf constituents by its solubility in a mixture of four parts of acetone and one of water, a solution which does not dissolve other plant substances.

The Nature of Photosynthesis.—When in pure solution chlorophyll does not show photosynthetic power. The efficiency of chlorophyll in its natural position is partly due to its occurrence in very thin layers. It has been shown that in sunlight a film of chlorophyll over a layer of gelatin, containing a tissue-extract, can cause fixation of carbon dioxide in the form of a substance giving tests characteristic of *formaldehyde*.

Bubbles of oxygen are also found in the layer of gelatin. The production of oxygen is characteristic of photosynthesis. It has long been known that animals can resist suffocation in a closed glass vessel in the sunlight if green plants are in the enclosure. The plant "fixes" the toxic

carbon dioxide produced by the animal and simultaneously liberates oxygen to sustain animal respiration. This is also the explanation of the success of the "balanced aquarium" in which stagnant water without aeration supports aquatic life if it is both plant and animal.

The fundamental reaction involved in photosynthesis may be represented in its essentials by the following equation.



This equation is in accord with the following facts: (1) Photosynthesis utilizes carbon dioxide and water apparently in equimolecular amounts; (2) molecular oxygen, in volume equal to that of CO_2 used, is produced; (3) carbohydrates which may be represented by the general formula $\text{C}_n\text{H}_{2n}\text{O}_n$ (*e.g.* glucose $\text{C}_6\text{H}_{12}\text{O}_6$) appear to be the first definitely recognizable product of photosynthesis.

When n in the above equation is one, formaldehyde (HCHO) is a product of the reaction. That formaldehyde might be the first stage in the photosynthetic process was suggested fifty years ago. Entirely satisfactory proof of this theory is difficult to obtain. Such proof must meet objections based upon (1) the common knowledge that formaldehyde in exceedingly small quantities (less than 0.001 per cent) is toxic to plants and (2) analyses showing that formaldehyde cannot be found in detectable quantities in plants. Both these objections are met by the highly probable assumption that formaldehyde, as fast as it is produced, is changed immediately into sugar by other reactions taking place in the plant. This assumption seems justified because living matter is known to effect similar syntheses. Such processes are called *Polymerization* or condensation, since a number of molecules of the same substance are condensed into one. The reaction for the polymerization of formaldehyde may be represented as: $6\text{HCHO} = \text{C}_6\text{H}_{12}\text{O}_6$. Other observations give confirmatory evidence for the theory. One of them is that the condensation of formaldehyde into sugar can be effected by chemical agencies in the laboratory. Another is that formaldehyde was detected in the gelatin-chlorophyll experiment described above. A third is the observation that formaldehyde in sufficiently dilute amounts may stimulate photosynthesis in plants in the sunlight but not in the darkness. There is good evidence, then, to support the formaldehyde-condensation theory.

Any theory to account for the nature of photosynthesis will be incomplete until the entire process may be artificially imitated and controlled. The fact that the process can not be imitated as yet is due to a circumstance frequently encountered in physiological research, namely: Life processes go on in complex stages. Changes, whether anabolic or catabolic, occur in a series of linked reactions. The process of formaldehyde production consists of several reactions involving chlorophyll and other constituents, and similarly the transformation of formaldehyde into sugar

probably occurs in several stages. The initial and final stages of such processes, sometimes a few of the intermediary ones, are often recognized easily; but difficult indeed is the task of completely unravelling the entire skein of interdependent reactions in a metabolic series. Such a chain of events, each one largely influenced by the progress of the preceding and succeeding ones, is made possible only by the peculiar and complex architecture of living matter. This is the most fundamental reason why chlorophyll on the surfaces of chloroplasts in a living cell behaves very differently from chlorophyll in a solution.

The Chemistry of Chlorophyll.—Extensive studies on the chemical composition of chlorophyll have thrown a little light on the origin of its remarkable properties. It is a complex organic compound, a derivative of the group known as **pyrrols**. It is related to some other natural pigments, among which is the red coloring matter, hematin of blood; but in contrast to chlorophyll, all other natural pigments, with the exception of certain red and brown ones of seaweeds, do not effect photosynthesis.

It has been shown that, as prepared by dissolving in acetone, chlorophyll contains magnesium combined in a form enabling it to be separated by treatment with acids. This fact indicates that chlorophyll probably exists in the leaf as a magnesium salt. The full significance of this fact is not apparent though there is, curiously enough, considerable evidence to show that both **magnesium** and **potassium** are required for photosynthesis. Inorganic ions, such as those of magnesium and potassium play an indispensable rôle in life processes. (See Chap. VI.) The particular rôle of certain ions has been recognized in some cases, so that the significance of magnesium and potassium for photosynthesis may be eventually established.

The green color of chlorophyll represents light waves which are not absorbed but are able to pass through or be reflected from the chlorophyll molecules. The *spectrum* obtained by passing sunlight or other white light through a living leaf or through a solution of chlorophyll and through a *spectroscope* shows distinctive dark bands or regions of absorption, none of which lie in the yellow or green regions of the spectrum.

The following brief description of the spectrum effect and spectroscope will serve as a review. For a fuller account a textbook of physics should be consulted. Light consists of electromagnetic vibrations. Though all electromagnetic vibrations are essentially of the same character, they vary greatly in wave length from the extremely long Hertzian waves, applied in wireless transmission, to the very short X-rays. It will be recalled that all transfer of energy across any space not permitting actual material contacts is by means of electromagnetic vibrations; so that studies of heat, light, ultraviolet radiation and X-rays, (all capable of producing marked effects on living matter) are applications of electromagnetic theory that are employed in physiology. The

vibrations capable of arousing sight in the human eye vary in length from about $400 \mu\mu$ (a millimu, $\mu\mu$, is $\frac{1}{1,000,000}$ of a millimeter), as found in the deepest violet light, to about $700 \mu\mu$, the extreme red rays. Sunlight is a mixture of these vibrations. Passed through a transparent prism or other refracting device, waves of different lengths are differently bent, so that the rainbow effect or spectrum is obtained. It is essentially a sorting out of the different rays. The spectroscope (Fig. 2) is an instrument for the production and observation of spectra. The prism form consists of three main portions: (1) The collimator, a device for bending the light by a series of lenses into a pencil of parallel rays, (2) a triangular

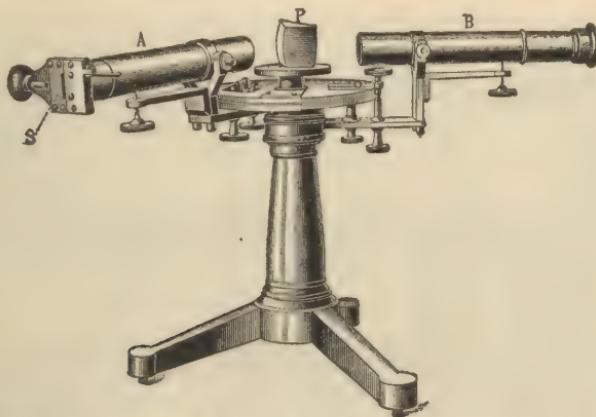


FIG. 2.—The spectroscope. *S*, the slit for admitting a narrow pencil of light rays. *A*, the collimator for producing parallel rays. *P*, the prism for refracting the light. *B*, the telescope for observing the spectrum. (*From Howell "Text Book of Physiology," W. B. Saunders Co.*).

prism of suitably chosen glass which causes the refractive sorting of the rays, and (3) a telescope device for observation of the magnified, focused image of the spectrum. The length of the band of color obtained will depend on the character, size and number of prisms used, in other words, upon the extent to which the light is dispersed by the refractive apparatus. Ordinary white light from an electric light filament or a luminous flame gives a continuous spectrum whose colors present rainbow tints merging from red to violet. Sunlight, however, shows the dark, so-called Fraunhofer lines, since certain of the lengths of light waves tend to be absorbed during the passage of sunlight through the atmospheric envelope of the sun. Just as the composition of the sun's atmosphere can be inferred from the positions of these dark line or absorption bands, so various substances in solution can be identified by the position of the dark bands in the spectrum given by white light after traversing the solution under examination. Positions of such bands may be described by reference to the Fraunhofer lines which have been designated for convenience by

letters of the alphabet, A, B, C, D, etc. or by statement of the actual wave lengths of the light whose absorption causes the dark bands. Since light absorption is due to peculiarities of molecular structure, every substance capable of showing an absorption spectrum is definitely characterized by its power to dampen or annul a certain assortment of light waves differing from the group absorbed by any other kind of molecules. The value of the spectroscope in qualitative analysis is apparent.

The spectra of chlorophyll under various conditions with the Fraunhofer lines for comparison, are represented in Fig. 3. These spectra show that chlorophyll absorbs chiefly light waves which correspond to the red and violet spectral regions. These are the light waves which

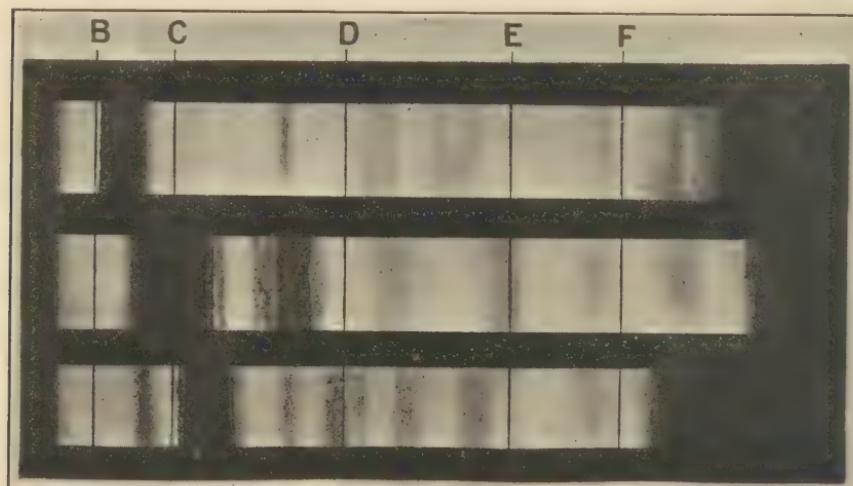


FIG. 3.—Spectra of Chlorophyll. The upper of the three spectra is that of chlorophyll in its natural state and is the one obtained when light is passed through the nettle leaf. The two lower spectra are those given by ether solutions of different modifications of "extracted" chlorophyll. (After Willstätter and Stoll).

most strongly affect photosynthesis as has been shown in a variety of ingenious experiments. Englemann devised an experiment in which bacteria of the aërobic type, which thrive only in the presence of an abundant supply of oxygen, were confined in an air tight space under a microscopic cover glass. Included in the culture fluid surrounding the bacteria was a slender filament of one of the aquatic plants belonging to the group of green algae. A spectrum of bright sunlight was then projected upon it, in the field of the microscope, in such a manner that the spectral colors were distributed along the filament. Those parts of the alga responding photosynthetically to the light would liberate sufficient oxygen to cause aggregation of the bacteria at such points. The typical result of an experiment is shown in Fig. 4. The Fraunhofer lines indicated in the figure may be used for reference. The figure shows that the regions of

active oxygen production, as indicated by accumulations of bacteria around the filament, correspond to the chief absorption regions in the chlorophyll spectrum. There are other experimental means for demonstration of the same fact. It therefore seems conclusively proved that only those light waves especially absorbed by chlorophyll stimulate the process of photosynthesis.

The great value of chlorophyll is this: It enables plants to convert carbon dioxide and water, which have no fuel value and therefore no energy value for life, into sugar, starch and other compounds which have a high fuel value readily available for development of energy in plant and animal organisms. Energy from the sun in the form of certain light rays is made available to life in the form of stored-up food. This is the fundamental fact of photosynthesis.

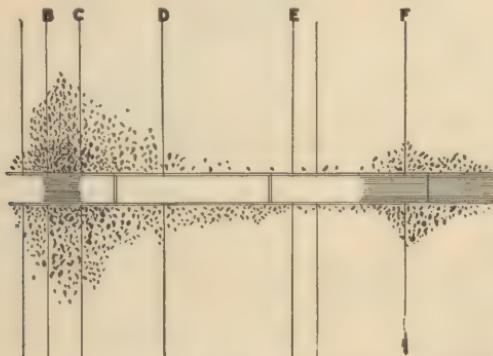


FIG. 4.—Diagram to show the results of Engelmann's experiment on the absorption of light by chlorophyll. The bacteria used in this experiment tend to accumulate at a source of oxygen. The dots show their accumulation at certain points along the green alga. They are the points illuminated by the spectral rays which chlorophyll absorbs. Reference to the Fraunhofer lines shown in the diagram will serve to locate these rays. As the emission of oxygen accompanies photosynthesis, the experiment indicates that this process is most effective under the influence of light rays that are absorbed by chlorophyll. (After Engelmann).

The Nitrogen Cycle.—Plants possess other important synthetic powers. Proteins, which will be discussed in Chap. II, have already been mentioned as the product of plant life. The metabolism involving proteins is measured practically by the utilization and excretion of nitrogen-containing compounds; since that element constitutes from $\frac{1}{4}$ to $\frac{1}{6}$ of the protein molecule and since over 90 per cent of the nitrogen actually present in living things occurs in the form of protein. The relationships of plants and animals to the utilization of nitrogen are not unlike those shown in a "carbon cycle." In Fig. 5 is a schematic representation of the "nitrogen cycle" taken from Bayliss.

It is seen that the great reservoir of nitrogen which constitutes about 79 per cent of the atmosphere is not available for use (fixation) by organisms other than the lowly forms of certain soil bacteria, known as the

nitrogen-fixers and those very interesting bacteria found in the **root nodules** of leguminous plants.

The activity of the nitrogen-fixers may be demonstrated by growing cultures of them in a solution containing no nitrogenous compounds but abundantly supplied with sugar. The bacteria grow and multiply and when a mass of them is subsequently collected and analyzed they are found to contain considerable quantities of nitrogen, chiefly in the form of protein, although atmospheric nitrogen was the only form of that element to which they had access. Atmospheric nitrogen is without fuel value or other energy content available for life processes. But proteins

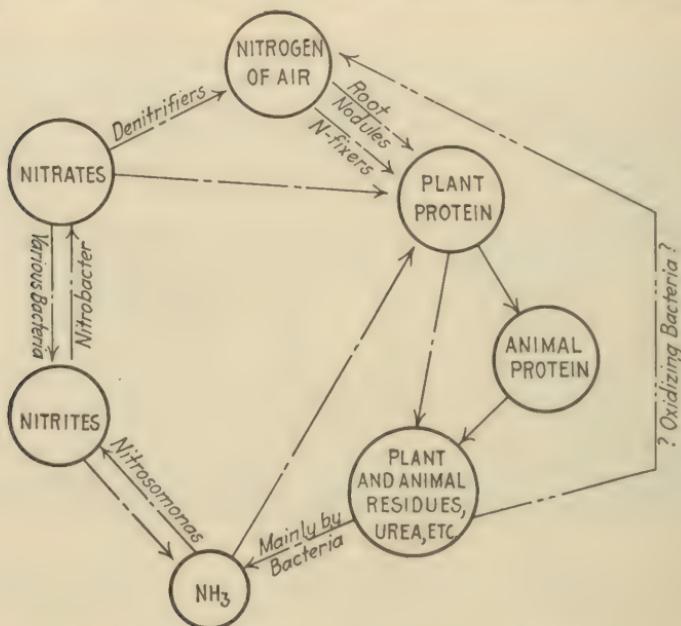


FIG. 5.—Diagram to indicate some of the ways in which a nitrogen cycle can occur in nature. (From Bayliss, "Principles of General Physiology," Longmans, Green and Co.).

have a high fuel value and other energy available for chemical activities especially those of metabolism. Energy has been stored in a new form. Where has it come from? The presence of sugar in the culture medium is requisite for synthesis and as the destruction of sugar during this process is proportionate to the production of protein, sugar is evidently the source of the energy which makes this kind of nitrogen fixation possible. As the supply of sugar or equally available fuel for bacterial consumption is not abundant in soil it is probable that nitrogen fixation by ordinary soil bacteria is of minor importance in the economy of nature.

A second type of nitrogen fixation consists of the activity of root nodules. Such structures occur commonly on leguminous plants: Peas, beans, clover, alfalfa, vetches, etc. It has been known since before

the Christian Era that one especially profitable type of rotation of crops consisted in planting a leguminous crop on a field to be used the following year for a grain crop. In other words soil is enriched by growth of legumes. Something of value is taken from the air. It is only in recent times, however, that the nodules upon the roots of legumes have been identified as the source of this valuable power. Sketches of the nodules are shown in Fig. 6. It has been further shown that microorganisms characterized by formation of branched structures called "bacteroids" are the active agents. (See Fig. 7.) They are widely distributed in soil and as they can penetrate into the root hairs of legumes where they multiply to form colonies they cause the nodule formation. The inter-

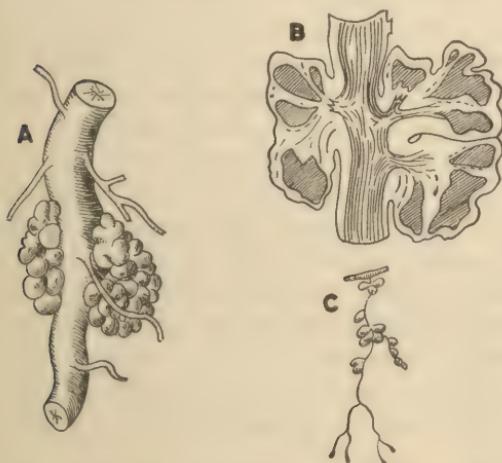


FIG. 6.—Root Nodules. A, nodules on a root of lupin. B, section of part of A. C, nodules on a root of clover. (A and B after Woronine, C after Vuillemin).

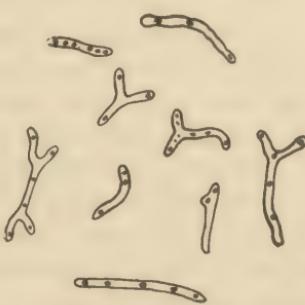


FIG. 7.—Bacteroids as seen in pure culture from nodules. (After Dawson).

mediate stages in the synthetic process are unknown; but the fact that leguminous roots with nodules enrich the soil with some sort of nitrogenous compounds available for all plants is well established. The energy required for the process is doubtless derived from substances produced by photosynthesis in the leaves of the legumes. We encounter here an example of a relationship frequently met with in nature—a sort of mutual benefit association of two living organisms. It is called **symbiosis**. In this instance the "bacteroids" aid the plant by synthesis of useful nitrogenous compounds while the legumes, since they alone appear to be capable of supporting active life of the microorganisms, must contain something peculiar in their juices which make them a suitable host for the "bacteroids."

Nitrates and ammonium compounds are suitable as a supply of nitrogenous food for nearly all plants and in fact constitute the only source of

nitrogen for the vast majority of green plants. Next to photosynthesis this is the most significant fact in the relation of plants to animals. Plants are the only source of protein foods for animals since even carnivores are indirectly dependent upon plant sources.

The residues of plants and animals are destroyed by various bacteria, some of which produce ammonia, while others appear to be able to liberate elemental nitrogen. (See Fig. 5.) Ammonia, however, may be indirectly transformed into atmospheric nitrogen and water by soil bacteria which yield nitrites and nitrates as intermediary stages. The bacterial activity changing ammonia into nitrites may be reversed by the activity of other bacteria as may also the transformation of nitrites into nitrates. Nitrates are the chief nitrogenous food for plants and because of this and because of the rapidity with which nitrates are transformed into nitrites on the one hand or reduced to yield atmospheric nitrogen on the other, only a small amount of nitrates can be found at any one time in soil or the ground waters of springs, wells, streams and lakes.

The analyst who utilizes chemical means for testing the purity of water depends very largely on determination of ammonium compounds, nitrites and nitrates for his information. The reason for this is obvious after consideration of the "nitrogen cycle." Either water is polluted with decaying organic matter and contains more or less ammonia and nitrites, the amount depending on the extent of pollution and the degree to which bacteria have disposed of it, or else it is comparatively free from ammonia and nitrites because it has been exposed during percolation through the soil to bacterial and other plant processes which have disposed of nitrogenous compounds.

Plant Synthesis of Fats.—Plants carry on all the basic synthesis of sugars, starches and related compounds, in short, of the carbohydrates. This synthesis may be defined roughly as the fixation of carbon dioxide. Furthermore plants carry on all the fixation of nitrogen into forms available for life, that is into protein and related materials. Besides the carbohydrates and proteins one other group, the fats, are commonly recognized as substances prominent among foods and important in the chemical make-up of organisms. Is fat production also dependent on plant life? Apparently not. There is no evidence to indicate that animals can not produce in their own bodies all the fatty materials they require provided their diet is not otherwise deficient. Osborne and Mendel, for example, have shown that rats could subsist during the entire period of rapid growth on a diet containing the merest traces of fat. Hindhede has made a similar demonstration on men. The well-known tendency of human beings and domestic animals to put on fat while subsisting on a diet rich in sugars and starches is an index of the ability of the animal organism to synthesize fat from carbohydrate. This can be proved, in fact, by satisfactory quantitative experiments as will

be explained in the chapter on fats. There are marked differences between the chemical constitution of the carbohydrate molecules and those of fats so that the ability to effect this transformation is an index of considerable synthetic powers in the animal organism in spite of its limitations in the matter of carbohydrate and protein synthesis. The vegetable fats such as olive oil, corn oil, peanut oil, cotton-seed oil and many others are useful in animal nutrition, but animals are not directly dependent on the plant kingdom for production of fats.

Plant Synthesis of Vitamins.—One other group of materials has been recognized in recent years as essential among the foods of living things. Substances called vitamins are now known to be absolutely indispensable to life. Here again the peculiar synthetic power of plants is preeminent over that of animals, for plants alone can produce vitamins. Animals subsisting on a diet, deficient in one or more of the three recognized vitamins, are subject to serious nutritional disturbances which may be fatal. A more detailed discussion of the vitamins will be given in Chap. VIII. It suffices for the present to emphasize the importance of plant synthesis for animal life. In the light of our present meager knowledge of the chemistry of vitamins, we are not able to explain the process of their production. It has been clearly shown that, in the case of two of the vitamins, seeds are relatively poor in their supply but early in the germination process when a young sprout two or three inches in length has formed, the latter is rich in vitamins. These appear to be produced from materials stored in the seed since the sprouting is brought about by merely soaking the seeds in tap water which contains no supply of foodstuffs other than traces of inorganic salts. In the case of one of the vitamins, at least, its production during the process of sprouting of oats or wheat is independent of photosynthesis. The vitamin is just as rich in sprouts obtained by germination in darkness as in sunlight. In the one case white or yellow sprouts are produced, in the other green ones; yet their vitamin content, as indicated by their ability to maintain good nutrition in experimental animals, is equal.

The Place of Plants in the Economy of Nature.—There are, then, at least three types of fundamental synthetic processes peculiar to plants: (1) Photosynthesis, resulting directly in the production of carbohydrate foods and permitting indirectly, through the use in living things of the solar energy thus stored, a wealth of living activities; (2) nitrogen fixation and other syntheses involving ammonium compounds and nitrates, resulting in the production of proteins which are absolutely requisite for life; (3) unknown synthetic processes which produce the vitamins, also indispensable for living things. The absolute dependence of animals upon plants is clearly emphasized. The biochemist conceives the words of the Psalmist, "All flesh is grass," in a very material sense. When one realizes the peculiar synthetic powers of plants, it is clear why agri-

culture is the basic industry of human life and why fertility of soil is the fundamental human wealth, the one indispensable human possession—its conservation and development the basis of all human progress.

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CHAPTER II

THE PROTEINS

Some knowledge of the basic materials of life, the stuff of which the vital mechanism is built and the fuel which it consumes, is essential at the outset of physiological studies. It is the alphabet of physiology. Of these materials proteins are the most widespread and the most interesting.

Proteins as the Most Complex of Physiological Materials.—The word protein, comes from the Greek verb meaning to take first place and was chosen because of the early recognition of the physiological importance of the group. The chemical composition of living matter is complex beyond anything we can hope to picture in the light of our present knowledge of chemistry. One first clearly realizes this fact when one undertakes a study of proteins which are found in all living matter. They are unquestionably the most complex compounds that have ever been investigated. Their enormous molecular weight is alone an index of this. Contrast, for example, the molecular weight of ordinary cane sugar with that of the protein, hemoglobin of blood. The sugar ($C_{12}H_{22}O_{11}$) has a molecular weight of 342, while hemoglobin, for which one estimate of its molecular formula is $C_{758}H_{1203}N_{195}S_3FeO_{218}$, corresponding to a molecular weight of 16,669, certainly has a molecular weight in the neighborhood of 15,000 or 16,000. The simplest of the proteins found in nature have molecular weights reckoned in thousands. So complex are the proteins that even the best chemical methods available for their study do not furnish the means for accurate determination of molecular weights and certain other attributes.

The most striking fact in regard to proteins is that they occur in every particle of living matter—are, indeed, indispensable to life.

Methods for Preparation of Some Representative Proteins.—The proteins vary considerably in their solubilities and other properties, so that a number of different methods have been employed to separate them from their natural sources. Since these methods reveal certain of the properties, characteristic of the different proteins, brief descriptions of a few representative preparations follow.

In muscle tissues, proteins comprise about 20 per cent of the muscle substance. As more than 70 per cent of muscle is water, protein is by far the most abundant solid matter. Muscle proteins will be discussed more in detail in a later chapter; but in this connection it is necessary to explain that, while nearly all the protein of living

muscle appears to be in solution, the larger part of the protein of dead muscle (meat) is insoluble in water and in all true solvents, that is, in all reagents which do not decompose it. The proteins, then, are altered at death.

This fact is typical of many which indicate one of the limitations to the study of biochemistry. It is very difficult to discover the composition of living matter, because when we subject it to any of the ordinary analytical processes we kill it and death produces profound chemical changes. We shall see in subsequent chapters the amazing ingenuity which biochemists have displayed in devising methods to overcome this difficulty and to make physiology what its name implies: The study of living things.

Of the "dead" proteins of muscle one is known as **myosin**. It is soluble in 5 per cent sodium chloride solution but insoluble in water or very dilute salt solution. It can therefore be prepared by thorough extraction of chopped meat with a 5 per cent salt solution and subsequent dilution with a large volume of water. The myosin settles out slowly as a flocculent white precipitate which may be filtered off.

From white of egg (albumen) the protein known as egg-albumin may be prepared. White of egg contains 12 per cent of protein, mostly **albumin**, about 0.7 per cent of inorganic salts and traces of sugar and some other organic compounds. When strained through cloth so as to break up the numerous filaments and thin membranes in it, white of egg, poured into ten times its volume of distilled water, yields a precipitate of a small quantity of the protein called **egg globulin**, which like myosin is insoluble in water or in very dilute salt solutions. The flocculent precipitate settles and may be filtered off giving a clear filtrate containing about 1 per cent of albumin uncontaminated by other protein and containing only small quantities of other substances. By evaporation of this solution at a temperature below that which coagulates albumin or by precipitation of albumin with alcohol the protein material may be obtained in solid form.

Of the several proteins in milk, **caseinogen** is by far the most abundant. It differs from the proteins, so far described, in the ease with which it may be precipitated from its solutions by slight acidification. If milk, preferably skimmed, is treated with a small amount of dilute acetic or hydrochloric acid, there is formed at just the right degree of acidity a heavy flocculent precipitate of caseinogen. It will redissolve in the presence of either a larger or a smaller concentration of acid. Like all protein precipitates, it tends to carry with it other substances present in the solution. Other milk constituents, proteins, fats, salts and milk sugar, are in the precipitate because they are "entrapped," as it were, when the caseinogen precipitate forms. To remove these adherent substances several manipulations are required. The caseinogen may be filtered off, suspended in 95 per cent alcohol in which it is insoluble, filtered, and then suspended in absolute alcohol and again filtered. The alcohol washings dehydrate it so that the next step, suspension in ether in which it is also insoluble, permits efficient solution of adherent fat in ether. After another filtration the caseinogen may be redissolved in a large volume of dilute ammonia and reprecipitated with dilute acetic acid to get rid of adherent proteins, sugar and salts. The solution and reprecipitation may be repeated several times if a high degree of purity is required. The precipitated caseinogen is again dehydrated with alcohol and washed with ether so as to yield a dry powder.

From wheat flour several proteins may be separated. If the flour is thoroughly stirred with cold water it tends to separate into (1) a gummy mass called **gluten**, which contains the wheat proteins, and (2) the minute starch grains which become suspended in the water. The water, poured off from the gluten, carries the starch grains with it. Repeated washing in this manner will eventually free the gluten from the starch. If the gluten is then put into a large volume of 80 per cent alcohol and heated at about 60°C. over a water bath for several hours, one of the proteins

of gluten, called **gliadin** is dissolved. Gliadin belongs to the group of proteins occurring in seeds and characterized by being soluble in 60 to 80 per cent alcohol in which most proteins are insoluble. After filtering the alcoholic extract of gluten, the filtrate contains gliadin which may be precipitated by dilution with a large volume of water or by addition of a large volume of alcohol. Gliadin is insoluble in sufficiently low or sufficiently high concentrations of alcohol. Filtered off and dehydrated by alcohol and ether washing, the gliadin is obtained in powder form.

One other method of preparation is of interest because it involves crystallization of a protein. Although a large number of proteins have been found to be crystallizable, the exact conditions, permitting crystallization in the case of most of them, are difficult to obtain. In a number of nuts and seeds, however, there occur proteins which crystallize under conditions readily obtained. From hempseed, for example, the protein, **edestin**, can be extracted by stirring the crushed seeds with 5 per cent sodium chloride solution, at 60°C. After filtering at the same temperature and allowing the filtrate to cool, beautiful tetrahedral and octohedral crystals of edestin are formed. It is much less soluble in 5 per cent salt solution at room temperature than at 60°C.

Other manipulations are employed in the separation and purification of the large number of different proteins that have been prepared from animal and vegetable substances. All the proteins, when isolated, are odorless, nearly tasteless powders and, with the exception of the red hemoglobin from blood and certain homologous blood proteins of invertebrates, are colorless.

The Elemental Composition of Isolated Proteins.—The elementary analysis of purified proteins shows that their composition varies, somewhat, as indicated by the following figures:

Carbon.....	50.0–55.0 per cent (about $\frac{1}{2}$ of the protein)	These 4 elements are found in all proteins
Hydrogen.....	6.5– 7.3 per cent (about $\frac{1}{15}$ of the protein)	
Nitrogen.....	15.0–17.6 per cent (about $\frac{1}{6}$ of the protein)	
Oxygen.....	19.0–24.0 per cent (about $\frac{1}{5}$ of the protein)	
Sulphur.....	0.3– 2.4 per cent (found in the majority of proteins)	
Phosphorus.....	less than 1 per cent (found in only a few proteins)	

In addition, small quantities of certain other elements, always less than 1 per cent, are found in some proteins of limited occurrence in nature. These include: Iron in hemoglobin of blood of vertebrates, copper in hemocyanin of blood of certain invertebrates, manganese and zinc in certain proteins of molluscan tissues, iodine in a peculiar and indispensable form as a component of a protein in the thyroid gland, and bromine in a protein substance of corals. Certain other substances, usually present in protein preparations, are not regarded as true protein constituents but rather as contaminations. They consist of inorganic salts such as sodium, potassium and calcium salts. Their presence is due partly to physical adherence and partly to a tendency of proteins to form salts with inorganic ions. Such contaminations are detected in the ash formed by burning the protein.

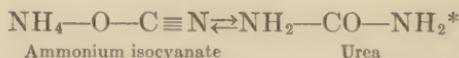
The Biochemical Significance of Carbon.—The large amount of carbon in proteins is significant. Carbon is the only element that forms com-

pounds as complex as proteins. This is due to the peculiar properties of the carbon atom. It has a valence of four and, since its properties are midway between those of the acid-forming elements and those of the base-forming ones, its four valences can be the points of attachment for almost any other element. Moreover, carbon can effect complex combinations of many of its own atoms. Long chains of carbon atoms, consisting of even fifty units, are recognized within the molecules of some organic compounds. Furthermore, any one of the carbon atoms in a chain may be the origin of a branch chain of carbon atoms or even of two branches. The individual carbon atoms are able to combine with each other in several different ways. One, two, three or even four of the valences may be utilized to bridge the gap between two adjacent carbon atoms. Carbon atoms can form strongly linked stable rings. Because of these chemical properties of the carbon atom, its combinations with oxygen, hydrogen, nitrogen, etc. in an innumerable variety of derivatives constitute the bulk of material in living structures. These peculiarities of carbon are among the things which make possible, as Henderson has pointed out, the existence of chemical combinations as complex in composition and in metabolic behavior as are those which constitute life as we know and understand it. A planet, composed of the recognized chemical elements but with no carbon available upon its surface for chemical activity would probably be lifeless. No other element displays the diverse reactivity of carbon.

The Distinction between Organic Chemistry and Biochemistry.—

Because of the peculiar properties of the carbon atom, the number of its compounds is beyond reckoning. For this reason their study has come to constitute a separate branch of chemical science. It is called Organic Chemistry. This name is misleading. It implies the study of the chemistry of organisms, that is, of living things. Although it does include the chemistry of very many substances occurring in organisms and their products, it also includes the study of many things not directly related to physiology. The name was chosen in the early part of the nineteenth century when the supposition prevailed that only living things could synthesize the carbon compounds. But when Wöhler in 1828 synthesized urea, a typical "organic" compound, by simply heating ammonium isocyanate, this supposition was proved to be erroneous.

The reaction occurring in Wöhler's experiment may be represented thus:



Ammonium isocyanate undergoes a molecular rearrangement and as it can be synthesized from the elements it is clear that urea may be produced by purely artificial means.

* The arrows pointing in both directions indicate that the reaction is reversible.

Many other carbon compounds, found in living structures and their products, have also been prepared since Wöhler's time by methods of laboratory synthesis. True, many physiological substances, including proteins, have not been prepared artificially. It must be confessed, indeed, that expenditure of much labor and experimental ingenuity has still left us far from possession of the means for artificial synthesis of proteins. The progress already made, as will be explained presently, is sufficient, however, to dispel the idea that all complex carbon compounds are exclusively the product of life.

Briefly defined, organic chemistry is the chemistry of the carbon compounds.

The chemistry of organisms requires a distinct name and so the word Biochemistry, the chemistry of life, is now used to refer to the subject matter originally included in Organic Chemistry. The two subjects greatly overlap since the bulk of biological material is carbonaceous. The organic chemist, however, deals with a host of substances not directly related to Physiology while the biochemist must study the physiological significance of many substances, notably inorganic salts, which do not contain carbon.

The Significance of Protein Nitrogen.—The nitrogen content of proteins is also significant. The atomic grouping of nitrogen in the protein molecule conveys important properties. A number of the varied reactions shown by proteins, including the power to combine with acids, are due to the nitrogen-containing groups. One has only to recall two very familiar compounds of nitrogen, ammonia and nitric acid, to appreciate the diverse and peculiar properties of this element.

Nitrogen occurs in different groupings in the protein molecule: (1)

|

Most of it is in the form of $-\text{NH}$, **the imide group**, with two valences of nitrogen attached to carbon atoms of other groups. In this form it can be separated only by comparatively intense chemical reaction which breaks the large protein molecule into smaller ones and eventually destroys it completely. The usual method of destroying the protein is to heat it with concentrated sulphuric acid at a high temperature. The nitrogen is then converted to ammonium sulphate. While such a destruction of protein in the laboratory requires a powerful chemical reagent acting at high temperature, a corresponding breakdown within the living organism occurs at ordinary temperatures in the ammonia producing bacteria (Chap. I). This contrast illustrates the great effectiveness of the chemical agents of living cells. (2) A smaller portion of protein nitrogen occurs as $-\text{NH}_2$, **the amino group**, with one valence of nitrogen attached to a carbon atom. In this form it is readily liberated in the form of elemental nitrogen by action of nitrous acid at room temperature.

THE CHEMICAL NATURE OF PROTEINS

Structural Formulas.—To both biologist and chemist the properties of any compound are almost completely summarized by a formula descriptive of the arrangement of atoms within the molecule. All chemical behavior, indeed, is dependent upon arrangement as well as upon the

kind and number of atoms present. Thus the formula, $\text{H}-\overset{\text{H}}{\underset{\text{H}}{\text{C}}}-\text{COOH}$,

for acetic acid conveys more information than does the mere empirical formula $\text{C}_2\text{H}_4\text{O}_2$ as determined by an elementary analysis for carbon, hydrogen and oxygen together with a molecular weight determination. The more extended or structural formula indicates that two carbon containing groups are present. Since such groups behave, under some conditions, as units that are transferred entire into other molecules, they are called organic radicals. It also indicates that one of the four hydrogen atoms is distinctly different from the others. As a matter of fact it can dissociate in watery solution to form a hydrogen ion which may be replaced by metallic or by basic radicals to form salts, the acetates. The other three hydrogen atoms are represented as attached to carbon in a uniform manner; indeed, acetic acid is often represented as CH_3COOH which is in agreement with the observed fact that three of the hydrogens behave in every way as though they were of equal value in the molecule. The group CH_3- is called the methyl group; $-\text{COOH}$ the carboxyl or organic acid group. The former is found with a hydroxyl group, $-\text{OH}$, in the molecule of methyl alcohol (CH_3OH) and since it has the alcohol forming power it is known, along with similarly behaving carbon groups (C_2H_5- , C_3H_7- , etc.), as an alkyl radical. The $-\text{COOH}$ group is the only form in which carbon can be so combined as to permit appreciable dissociation of the hydrogen ion. The properties of alkyl and carboxyl groups show, respectively, that carbon, like nitrogen, is capable of entering into both acidic and basic combinations, that is, $\text{H}-$ ion and $\text{OH}-$ ion forming substances.

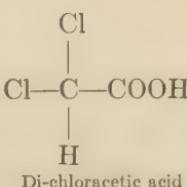
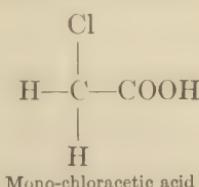
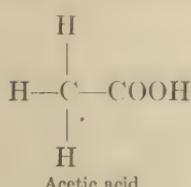
From the various chemical reactions which an organic compound may give it is possible to construct a complete picture of the internal arrangement of all its constituent atoms. The numerous and ingenious methods applied to such formula-making can not be described here but must be obtained from works on Organic Chemistry. Suffice it to say that two main types of methods are employed: (1) Studies of the decomposition products of compounds, a "chemical dissection" of the molecule, so to speak, and (2) attempts at artificial synthesis from substances of known molecular structure, analogous to the reconstruction of a picture puzzle from its fragments. How far such efforts have enabled us to peer into the intricate atomic grouping of the protein molecule will be shown in the following sections.

Hydrolysis.—The most instructive procedure for disclosing the nature of the protein is hydrolysis. From its origin the term obviously indicates water-splitting but it is also employed to signify the simultaneous cleavage of various molecules by the combined action of H⁺ and OH⁻ ions which arise, to be sure, as split products of water. Hydrolysis, in biochemical usage, always implies that larger molecules are broken into smaller ones. The actual reagents used include: (1) Cold water, which is hydrolytically effective in only a few cases; (2) boiling water, which is slightly more effective; (3) superheated steam, still more active; (4) dilute watery solutions of acids or alkalies, which by electrolytic dissociation increase the abundance of H⁺ or OH⁻ ions and are therefore especially useful hydrolytic agents and (5) hydrolytic enzymes, such as pepsin and trypsin of the vertebrate digestive system.

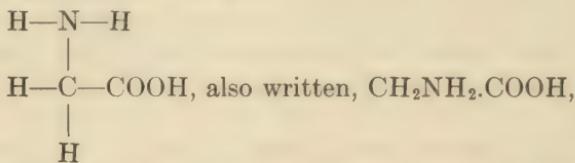
Amino-acids, the Product of Protein Hydrolysis.—If carried to its completion, hydrolysis invariably gives the same result on a given protein no matter which one of the various hydrolytic agents is used. Long continued application of superheated steam or prolonged boiling with 10 per cent sulphuric acid, or with 30 per cent hydrochloride acid or long continued action of trypsin all give the same assortment of products which may be separated and identified. They are called **amino-acids**. Even when oxidation accompanies hydrolysis, as in the action of hot potassium hydroxide or nitric acid, oxidation products of the same amino-acids are formed. As diverse agencies produce identical split products, it seems altogether likely that the fragments represent atomic groupings which exist in the original protein.

Emil Fischer, who has been a prominent pioneer in protein investigations has called the amino-acids the “building stones” of the proteins. One can conceive of proteins as built up in the anabolism of plants and animals by the union of amino-acids somewhat as a house is constructed of bricks or building stones. It is therefore obvious that a chemical explanation of the nature of proteins should begin with a consideration of the amino-acids.

The term, amino-acids, signifies that these compounds are amino derivatives of organic acids. The meaning of the phrase, derivatives of organic acids, is indicated by the following examples of such derivatives. Compounds, produced from acetic acid by substituting chlorine in the place of one or more of the hydrogen atoms in the methyl group, can be formed by passing chlorine gas into boiling acetic acid, containing a trace of iodine. The substitution is represented thus:

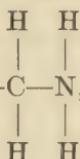


These substitution products are, then, chlorine derivatives of acetic acid. Similarly, amino-acetic acid:



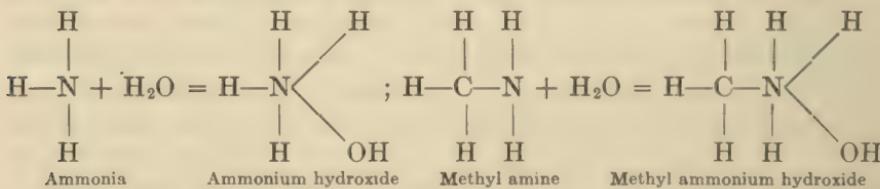
which is formed by treatment of mono-chloracetic acid with ammonia, may be regarded as a substitution product. In this case, the amino group ($-\text{NH}_2$) replaces one of the hydrogen atoms of the methyl group. Amino-acetic acid is commonly known as **glycocol** (from the Greek word for sweet, since it has a sweetish taste) or **glycine** and is one of the most abundant and widespread of the amino-acids that are the "building stones" of proteins.

The amino group is, as its name suggests, related to ammonia. The group is, indeed, a fragment of the ammonia molecule, transferred to another compound. There is a long series of compounds, called amines, which are all derivatives of ammonia. An example of them is methyl

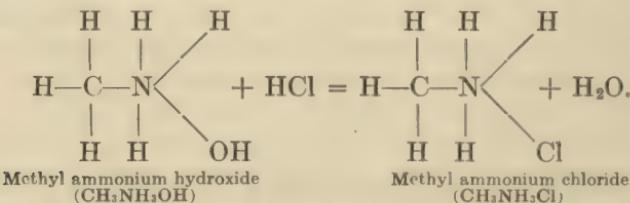


amine: $\text{H}-\overset{\circ}{\text{C}}-\text{N}$, or CH_3-NH_2 . The amines have properties similar

to those of ammonia. They are volatile bases with an ammoniacal odor. They form hydroxides with water, simultaneously changing the valence of nitrogen from three to five, in the same manner that ammonia does.

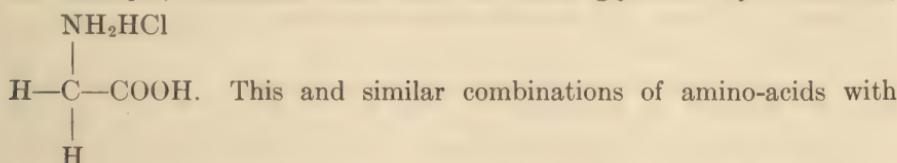


The resulting hydroxides combine with acids in the same way that ammonium hydroxide does.



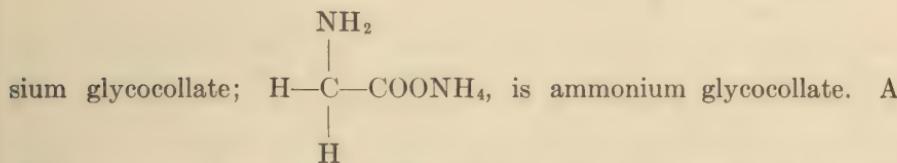
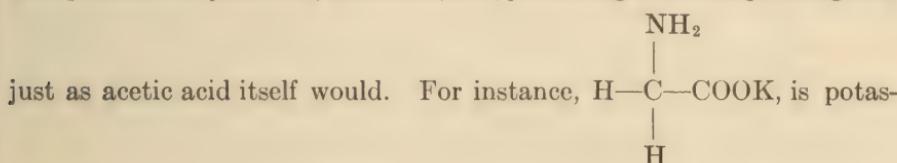
Just as the amino group confers the properties of ammonia to the amines, so it similarly affects amino-acids. An amino-acid retains the basic

action of ammonia, so that it can combine with other acids. Glycocol, for example, combines with HCl to form glycocol hydrochloride,

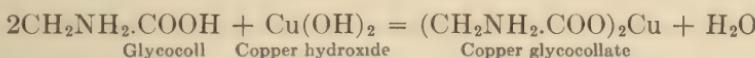


other acids are just as well defined, crystallizable compounds as is ammonium chloride or glycocol itself.

The amino-acid, then, is a base; but it is, nevertheless, an acid. It can combine with any basic substance. Glycocol, for example, reacts with potassium hydroxide, ammonia, etc., producing the corresponding salt,



salt of glycocol, well known because it is readily crystallized, is copper glycocolate.



This power of being either an acid or a base is the outstanding characteristic of amino-acids. It gives them a remarkable value in vital activities since it enables them to enter into many different chemical reactions. Substances possessing this property are called **amphoteric**. Amino-acids are not unique in this respect but they do hold a very prominent place among the amphoteric substances of organisms.

Separation of the Various Amino-acids Derived from Proteins.—When the proteins are broken down by any of the various hydrolytic agents, a mixture of different amino-acids is obtained. Isolation of the individual compounds may, in the case of some of the amino-acids, be obtained by mere crystallization. The less soluble ones crystallize from a solution of the mixed amino-acids so that they may be filtered off and, by recrystallization, obtained in pure form. The first amino-acid to be discovered, the one called **tyrosine** (from the Greek word for cheese) was isolated in this way. Enzymes derived from the moulds which cause the "ripening" of cheese hydrolyze casein and other milk proteins to produce amino-acids, so that a hot water extract of old cheese, on

partial evaporation and cooling, deposits well-formed crystals of tyrosine together with other amino-acids. By recrystallization pure tyrosine is obtained. Similarly, other amino-acids have been isolated by means of fractional crystallization.

For more extended knowledge of the amino-acids a method of more general application was needed. Emil Fischer's ester distillation method met this need. The principles involved in his method may be briefly outlined. A mixture of amino-acids obtained by the hydrolysis of any protein in boiling hydrochloric acid is suspended in absolute alcohol and subjected to a stream of bubbles of hydrochloric acid gas. Under these conditions, the amphoteric amino-acids attach hydrochloric acid to the amino group and the alkyl radical of alcohol (ethyl) to the acid group, forming what are known as hydrochloric acid esters. In this form they are volatile enough to be distilled and as they volatilize at different temperatures and pressures, it was possible for Fischer to devise a process for separating them. He employed liquid air to condense the more volatile substances and a high heat *in vacuo* to volatilize the heavier esters. By such manipulations the various esters were separated into a number of fractions. Each fraction contained only the small number of amino-acids whose esters possessed approximately the same volatility. Each fraction was redistilled to yield smaller fractions of which those of approximately the same boiling point were united. Sufficient repetition of fractional distillation eventually yielded products of constant boiling point, an index of chemical purity. Esters so obtained can be converted by boiling with water into the several amino-acids which may be crystallized or converted into some salt that can be more conveniently crystallized. The mechanical details of this method are obviously complicated and the technical skill involved in its manipulation is considerable. Only a limited number of investigators have employed it.

By it and some other methods, however, eighteen different amino-acids have been obtained, studied thoroughly and proved to be preformed constituents of proteins. For each of the eighteen, the chemical behavior and the products of decomposition by oxidation and other procedures are well known. Results of such investigations indicate the molecular structure and since all have been produced by artificial laboratory synthesis from substances of known structure, the molecular pattern of each of the eighteen has been definitely proved.

Two others which occur in small amounts have been distinguished. Their structures have not yet been definitely mapped. Whether still others exist in nature is questionable. If so, they form only a small part of protein molecules. A comparison of the total nitrogen of the proteins, which have been extensively studied, with the total nitrogen of the several constituent amino-acids shows that not far from 90 per cent of the protein molecule is accounted for. Unavoidable losses of known

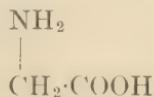
amino-acids, during separation and identification, doubtless account for at least a part of the missing contingent.

The detailed structural formulas and chemical and physical properties of all amino-acids need not be memorized in order to understand their significance for protein chemistry and for physiological processes, but a certain familiarity with some of them is almost indispensable. *Glycocoll, leucine, alanine, phenylalanine, tyrosine, lysine, arginine, histidine, aspartic acid, glutamic acid, proline, cystine and tryptophane* are names so frequently employed in any discussion of the nutritive requirements of animals or the metabolism of all living things that these names are an essential part of the physiologist's vocabulary.

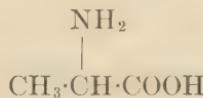
Students of organic chemistry to whom structural formulas convey a shorthand description of the properties and behavior, in short, the chemical attributes of compounds so represented, will find the following list a helpful review and summary.

A. Monoamino-monocarboxylic acids.

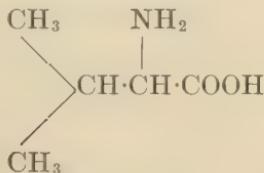
1. Glycine, (glycocoll) amino-acetic acid:



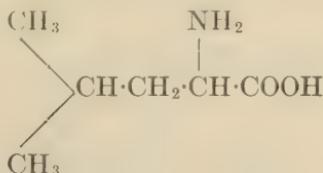
2. Alanine, α -amino-propionic acid:



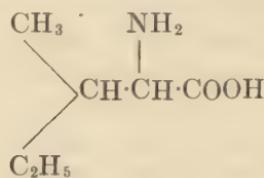
3. Valine, α -amino-iso-valerianic acid:



4. Leucine, α -amino-iso-caproic acid:



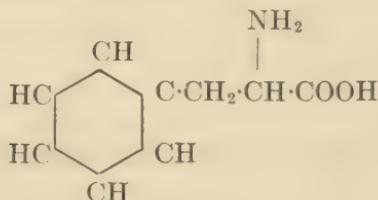
5. Isoleucine, α -amino- β -methyl- β -ethylpropionic acid:



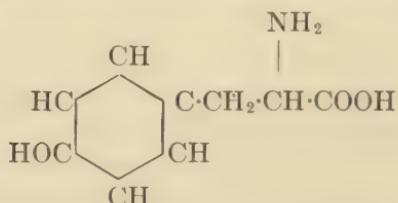
6. Caprine or glycoleucine, α -amino-normal-caproic acid:



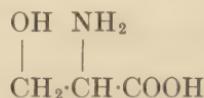
7. Phenylalanine, α -amino- β -phenyl-propionic acid:



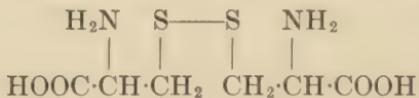
8. Tyrosine, α -amino- β -parahydroxyphenyl-propionic acid:



9. Serine, α -amino- β -hydroxypropionic acid:

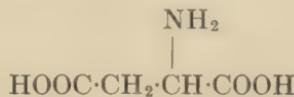


10. Cystine or dicysteine, di-(α -amino- β -thio-propionic) acid:

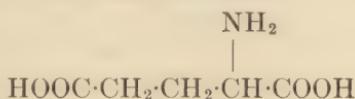


B. Monoamino-dicarboxylic acids.

11. Aspartic acid, amino-succinic acid:

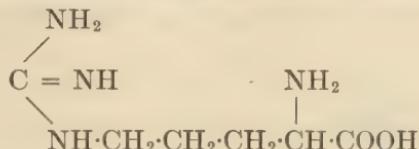


12. Glutamic acid, α -amino-glutaric acid:



C. Diamino-monocarboxylic acids.

13. Arginine, α -amino- δ -guanidine-valeric acid:

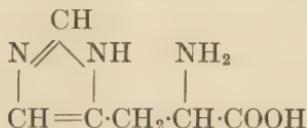


14. Lysine, α - ϵ -diamino-caproic acid:

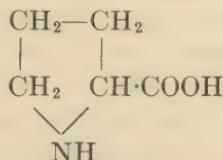


D. Heterocyclic amino-acids.

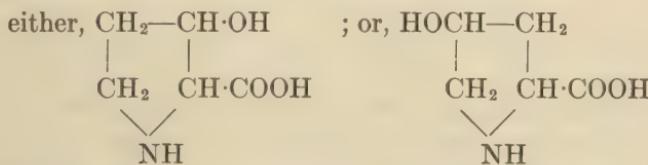
15. Histidine, α -amino- β -imidazole-propionic acid:



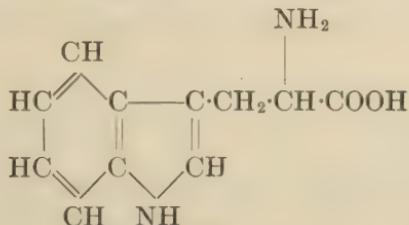
16. Proline, pyrrolidine-carboxylic acid:



17. Oxyproline, hydroxy- α -pyrrolidine-carboxylic acid:

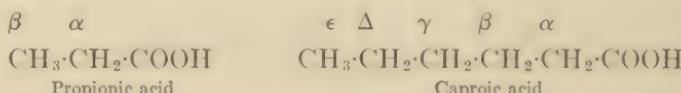


18. Tryptophane, α -amino- β -indole-propionic acid:



Some points of general interest in considering the structure of these eighteen compounds are the following. (1) The carbon chains constituting the molecular framework of the various organic acids of which they are derivatives vary in length from the two-membered one of acetic acid in glycine to the six-membered chain of caproic acid in caprine and lysine. (2) The chains are branched in leucine and isoleucine. (3) Two amino-acids contain the so-called benzine ring in the form of the phenyl group. (4) Some contain complex rings, composed of both carbon and nitrogen, and hence called heterocyclic rings. These occur in histidine, proline, and tryptophane. (5) The majority of the amino-acids contain one amino group and one carboxylic group and are consequently about equally strong in their basic and acidic behavior; two, aspartic and glutamic acids, have one amino group and two carboxylic groups so that they are predominatingly acidic in their reactions; three, arginine, lysine and histidine, contain two or more nitrogenous groups and therefore show more markedly basic than acidic character. These three have been called the "hexone bases."

One structural feature is common to all the amino-acids: An amino ($-NH_2$) group is attached to the carbon atom standing next to the carboxyl group. Although proline is an apparent exception to this rule, yet the imino ($-NH-$) group which here takes the place of an amino group is arranged with one bond held by the carbon atom next to the carboxyl. Now the several carbon atoms, which constitute the framework of any organic compound, do not have the same relation to the compound as a whole; so that attachment of a derivative group to different carbon atoms produces different properties in the resultant substances. For example, the substance represented by the formula $CH_2(NH_2)\cdot CH_2\cdot COOH$ is not identical with alanine, $CH_3\cdot CH(NH_2)\cdot COOH$, although either of them is properly considered to be amino-propionic acid. Substances composed of the same atoms in different arrangements are called chemical isomers. To distinguish their several forms of structure it is necessary to show which carbon serves as point of attachment of derivative-forming groups. For conciseness, then, the carbon atoms constituting a chain in any organic molecule are designated in order by the first letters of the Greek alphabet beginning with the carbon atom next the distinctive, characteristic group of the compound. For organic acids the carboxyl group is obviously the characteristic one, so that the carbon atoms are designated thus:



Alanine is called, therefore, α -amino-propionic acid while lysine is $\alpha\text{-}\epsilon$ -diamino-caproic acid. Every naturally occurring amino-acid contains

an amino group or the corresponding imino group in the α position. As other forms of amino-acids can be produced by artificial synthesis, it is a curious fact that all naturally occurring ones are of the α type alone.

It is also striking that of the scores of known organic acids, many of which occur in nature, only a few are found as amino derivatives in substances of biological origin. The proteins themselves are almost infinite in variety yet they are all constructed out of a comparatively small number (perhaps not more than twenty) unit "building stones."

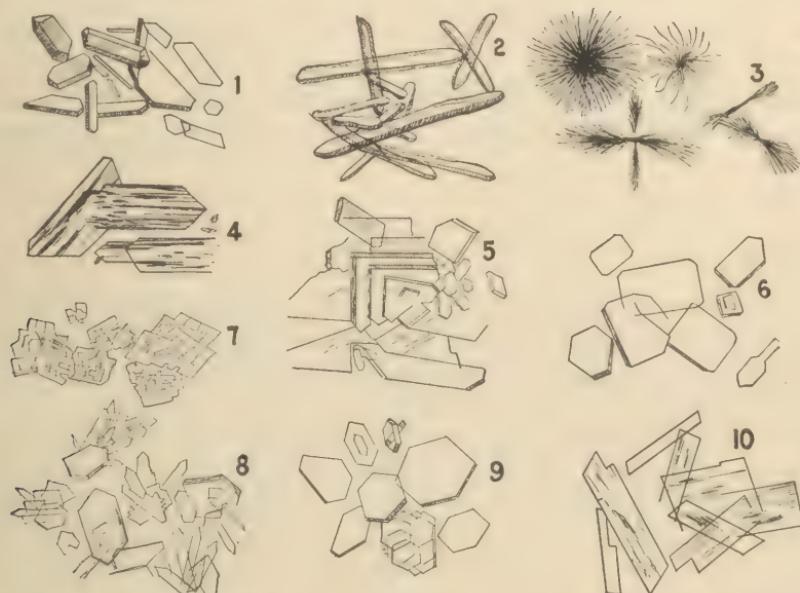


FIG. 8. Sketches to show the microscopic appearance of amino-acid crystals. 1, Glycocol. 2, Glyeocoll ethyl ester hydrochloride. 3, Tyrosine. 4, Glutamic acid. 5, Aspartic acid. 6, Serine. 7, Phenylalanine. 8, Leucine. 9, Cystine. 10, Copper salt of proline.

The general properties of amino-acids are not especially characteristic. All may be prepared in the form of white powders. All may be crystallized, though the majority have been obtained only as microscopic crystals. They are odorless and, with a few exceptions, tasteless. Their amphoteric behavior, the most significant property, has already been described. It results in a wealth of possible salt formation. Sketches of crystals of various amino-acids or their salts in crystal form are given in Fig. 8.

The assortment of amino-acids, obtained after hydrolysis of proteins, varies with the character of the protein used. Some proteins reveal a complete lack of certain amino-acids and only a comparatively small number of proteins can be credited with a full representation of the entire list. The quantitative yields of the amino-acids also show wide

variation. Arginine is found to the extent of 87 per cent in a protein obtained from the spermatozoa of salmon while it constitutes less than 8 per cent of gelatin. Glutamic acid is found to compose 43 per cent of wheat gliadin but less than 2 per cent of gelatin. The table given below will serve to indicate the qualitative and quantitative differences among the amino-acid yields of various representative proteins. Information of this character has been of great practical significance in the science of

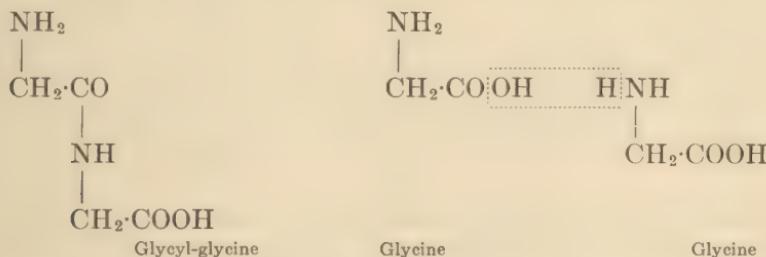
THE DIFFERENT AMINO-ACID YIELDS FROM VARIOUS REPRESENTATIVE PROTEINS
Figures are in per cent

Name of amino-acid	Casein of milk	Gliadin of wheat	Zein of corn	Gelatin from bone	Proteins of beef	Proteins of chicken	Salmin from sperm	Clupein from sperm
Glycine.....	0.0	0.0	0.0	16.5	2.06	0.68		
Alanine.....	1.5	2.0	9.8	0.8	3.73	2.28		+
Valine.....	7.2	3.34	1.9	1.0	0.88		1.65	+
Leucine.....	9.4	6.6	19.55	7.1	11.65	11.19		
Proline.....	6.7	13.2	9.04	9.5	5.82	4.73	4.3	+
Phenylalanine.....	3.2	2.35	6.35	1.4	3.15	3.53		
Aspartic acid.....	1.4	0.58	1.71	3.4	4.51	3.21		
Glutamic acid.....	11.0	43.7	26.2	5.8	15.49	16.48		
Serine.....	0.5	0.13	1.02	0.4			3.25	+
Tyrosine.....	4.5	1.5	3.55	0.01	2.2	2.16		
Arginine.....	4.84	2.7	1.55	8.2	7.47	6.34	89.2	88.0
Lysine.....	5.95	0.6	0.0	5.9	7.59	7.24		
Histidine.....	2.5	1.5	0.82	0.9	1.76	2.47		
Tryptophane.....	1.5	1.0	0.0	0.0	+	+		
Cystine.....	0.07	0.45	0.0	0.0	+	+		

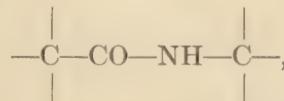
animal nutrition. Since animals require a number of amino-acids presented to them preformed, foods are adequate for an animal only when their content of amino-acids is able to meet the needs of the individual. The fact that the various amino-acid yields in no case add up to 100 per cent of the protein shows that our knowledge of the components of even the most thoroughly investigated proteins is still somewhat incomplete.

Attempts at Artificial Synthesis of Proteins.—A complete understanding of the nature of any organic compound should include successful artificial synthesis. Attempts to produce synthetic proteins, though unsuccessful in forming any of the natural ones, have thrown much light on the structure of the protein molecule. The varied and ingenious methods used need not be described here. They may be found in works on organic chemistry. By these synthetic methods, the union of amino-acid molecules with each other has been effected. The nature of

the union is shown in the formula of glycyl-glycine which is, in effect, a combination of two molecules of glycine with the elimination of one molecule of water. Thus:



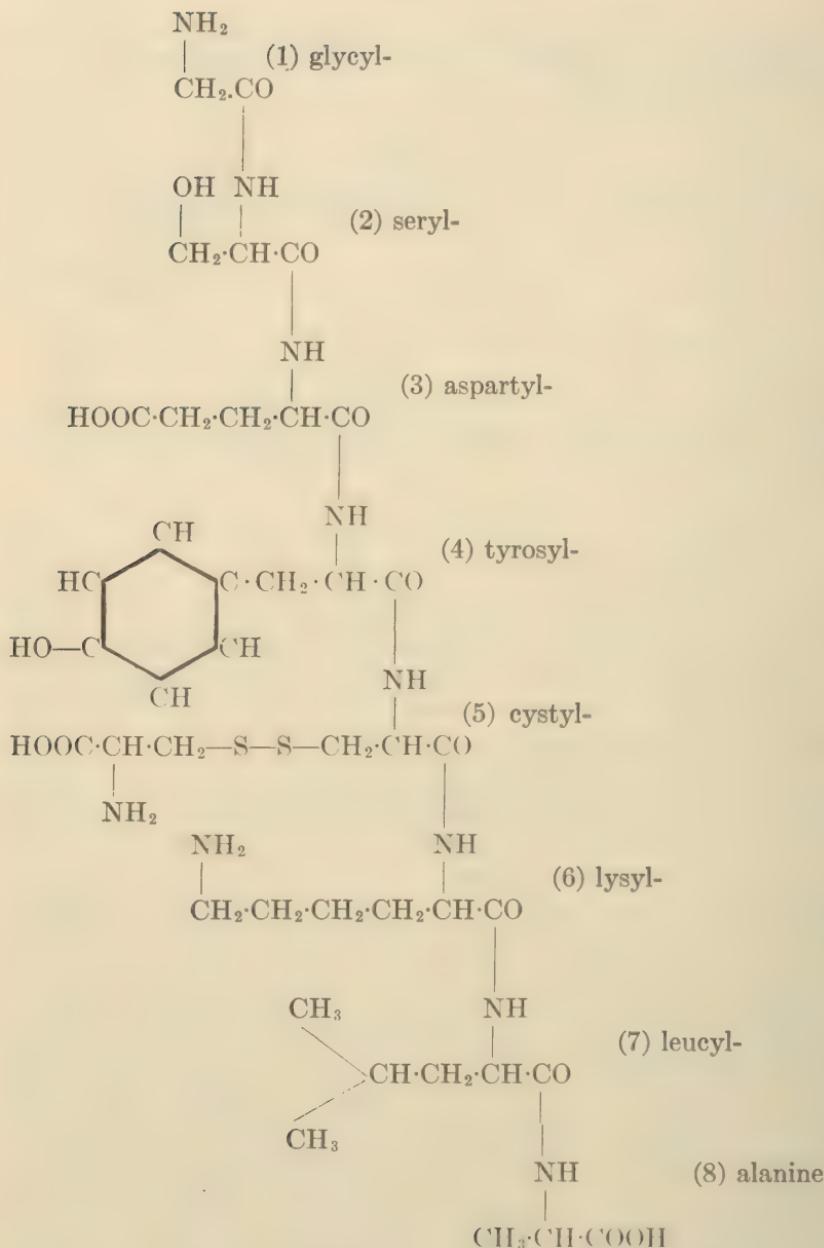
Glycyl-glycine contains $-\text{CO}-$ of a carboxyl group united to $-\text{NH}-$ of an amino group. Since $-\text{NH}-$ is called an imide group the union formed thus,



is called the imide linkage. It consists, essentially, of a union of the amino group of one amino-acid with the carboxyl group of another. Substances consisting of amino-acids combined by means of imide linkage are called **peptides**. If composed of two amino-acid residues they are **dipeptides**, of three, **tripeptides**, etc. If many amino-acids are condensed into one molecule the result is called a **polypeptide**. One of the artificial peptides obtained by Fischer was produced by the union of 18 molecules of amino-acids: 15 of glycocoll and 3 of leucine. Others of equal or greater complexity have been made.

Such peptides have many of the properties of proteins. With a molecular weight reckoned in thousands, they give a colloidal behavior which, as will be shown below, is characteristic of natural proteins. They are precipitated by some of the same reagents which precipitate proteins. The peptides are broken down to their constituent amino-acids by various hydrolytic agencies including the protein-digesting enzyme of the intestine. The similarities between these artificial products and natural proteins are sufficiently encouraging to make it seem possible that a proper combination of methods, already available, might produce a true synthetic protein. The work has at least made it clear that the imide linkage of amino-acids, as it exists in polypeptides, is also a feature of proteins. That amino-acids are linked together in the protein by other than the imide arrangement is highly probable; but in the main, the protein structure is that of the polypeptide on a very large scale.

In consequence of the imide linkage, the diamino-acids in a protein molecule require only one amino group for attachment to adjacent groups and may therefore furnish one free amino group to the protein.



(Formula of a hypothetical peptide to show the imide linkage such as exists in protein molecules between the several constituent amino-acids. Although the majority of amino and carboxyl groups are masked, those at the terminations of the chain and those furnished by diamino—or dicarboxylic—acids are free, so that they may be expected to confer amphoteric properties upon the compound as a whole.)

The number of free amino groups in any protein can be measured by the nitrous acid reaction mentioned above. The measure of the amino groups, thus obtained in the case of certain proteins, corresponds closely to the proportion of diamino-acids to be found among the decomposition products of the same proteins. This finding indicates that only the diamino-acids enable a protein to possess free amino groups while the α -amino groups are masked in imide linkings. Correspondingly, the basic character of any protein is roughly proportioned to its content of diamino-acids.

In the imide linkage the dicarboxylic amino-acids, namely aspartic and glutamic acids, would furnish free acid groups to the proteins. It is, indeed, noticeable that proteins containing especially large proportions of these constituents have markedly acid behavior.

It is to be expected that if amino-acids are linked in straight chain formation, such as is represented in the polypeptide formula shown on page 32, there would be at least one terminal —NH₂ group and one terminal —COOH group free to show their respective basic and acidic properties in the protein. The terminal groups are not the only combining ones, however, because proteins have been shown to be able to combine with more than one molecular equivalent of either acids or bases. Proteins, like amino-acids, are markedly amphoteric.

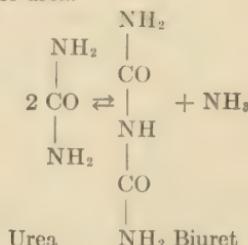
A Descriptive Definition of a Protein.—The chemical nature of proteins is summarized in the following descriptive definition. Proteins are compounds of high molecular weight containing carbon, nitrogen, hydrogen and oxygen, usually containing sulphur, sometimes phosphorous and rarely other elements; their structure is essentially a condensation of many molecules of various amino-acids, united, for the most part, by the imide linkage; proteins have an amphoteric reaction and possess free amino and carboxyl groups; by any type of hydrolysis, proteins yield amino-acids but the yield from different proteins varies both qualitatively and quantitatively.

Chemical Properties of the Proteins.—The solubilities of different proteins vary so widely that no general statements concerning them are of value. The solubilities are, indeed, so different that a convenient classification is based largely upon them. The more important variations in solubility are therefore given under the descriptions of the different classes of proteins in the next section.

A number of so-called color reactions of the proteins have assumed considerable importance for qualitative detection and since each reaction is given by certain constituent groups of the protein molecule, valuable information as to the components of specific proteins may be obtained in such tests.

The biuret test, one of the color reactions, is performed by making a protein solution, or the solution surrounding a suspension of insoluble protein, strongly

alkaline with potassium or sodium hydroxide and then adding, drop by drop, a solution of copper sulphate previously diluted until it shows the merest trace of color. A positive result consists in the production of a violet color of the solution, if the protein employed is soluble, or a violet color of the suspended particles of an insoluble protein. The blue color of copper hydroxide is formed in a negative result or in the presence of an excess of copper. From a study of peptides it has been found that any compound consisting of a union of four or more amino-acids by the imide linkage will respond positively. The biuret test may be regarded, therefore, as confirmatory evidence of the imide grouping of amino-acids in the protein molecule. The test is given by a number of non-protein substances. Its name, indeed, refers to the fact that the test is given by the compound called biuret which arises, together with ammonia, upon dry heating of urea.



A number of substances besides proteins, polypeptides and biuret respond with a violet color in this test. They all have one characteristic in common, namely a complex chain of carbon and nitrogen atoms. Obviously a positive biuret test does not by itself prove the presence of protein and the usefulness of the test is further restricted by the necessity for absence of interfering colors where a delicate violet is to be detected. The reaction is very useful in one way since all proteins give it even in high dilution; so that when it is possible to demonstrate a negative result for the biuret test, complete absence of proteins may be assumed. The progress of enzyme digestion or other hydrolysis of protein, for example, may be followed and completion of the process recognized by use of the biuret test. The term "biuret-free" is used as practically synonymous with protein-free.

The **xantho-proteic test** (xantho—from Greek word for yellow) is made by boiling proteins, either solid or in solution, with concentrated nitric acid so as to produce a clear yellow solution which, after cooling, is treated in a test tube with ammonium hydroxide poured in while the tube is inclined so that ammonia remains in a layer, stratified above the nitric acid-containing solution, with a neutral zone forming between the two layers. A positive result is a deep orange color in the alkaline layer and a white precipitate in the neutral zone, while the nitric acid layer retains a yellow color. The colors have been proved to be due primarily to the presence of the benzine ring, an atomic group which constitutes part of the molecule of phenylalanine, tyrosine and tryptophane. A positive result in the xanthoproteic test applied to a protein is therefore proof that among its "building stones" one or more of these three amino-acids may be found. The very general response of proteins to the test shows the widespread occurrence of the benzine grouping among natural proteins.

Millon's test is made by treating proteins, either solid or in solution, with Millon's reagent (a mixture of the several nitrates of mercury in nitric acid solution) and boiling. Protein if present is precipitated by the reagent and the particles of protein turn to a brick red color during the boiling. This test is given by all compounds containing the hydroxy-phenyl group, $\text{C}_6\text{H}_5-\text{OH}$. The simplest of such compounds is phenol, carabolic acid, $\text{C}_6\text{H}_5-\text{OH}$, which gives a brilliant red color with Millon's reagent without heating. Tyrosine is the only one of the amino-acids containing this

group. Since pure tyrosine gives the test and only proteins which yield tyrosine among their hydrolytic products respond to it, a positive result in Millon's reaction may be taken as proof of the presence of tyrosine among the constituent groups of a protein. Most proteins give the test. Gelatin and the proteins of spermatozoa are the notable exceptions.

The **glyoxylic acid test**, also called the Hopkins-Cole test, is made by treating a protein solution, to which a small amount of glyoxylic acid has been added, with concentrated sulphuric acid. A violet color is produced. The test is usually made by stratifying the protein solution above the sulphuric acid in a test tube so that the color appears in the zone between the strata. This reaction has been shown to be due to the indol group which is a component of the amino-acid, tryptophane. A protein which responds positively to this test is therefore shown to contain tryptophane among its "building stones." The occurrence of tryptophane in proteins is, in general, similar to that of tyrosine so that only a few protein materials fail to give the glyoxylic acid test.

The actual color-producing reactions occurring in the two last described tests are not entirely understood; but clearly a profound hydrolysis, in the one case by boiling nitric acid and in the other by concentrated sulphuric acid, is required for liberation of the protein groups entering into the color production.

A number of other protein color tests may be found described in laboratory manuals. The four above are the most widely used.

Precipitation reactions of proteins are very numerous. Among the substances that are general protein precipitants are: (1) Concentrated *mineral acids*, nitric, hydrochloric and sulphuric; (2) *salts of the heavy metals* especially mercuric salts. Copper, iron and lead salts when added in the correct concentration cause precipitation of most proteins but when added at higher or lower concentration do not precipitate. (3) The *alkaloidal reagents*, so called because they precipitate the vegetable, nitrogenous, basic substances called alkaloids, are general precipitants for proteins. Such reagents are: Picric acid, chromic acid, tannic acid, phosphotungstic acid, potassium-mercuric iodide, phosphomolybdic acid and trichloracetic acid. With several of the alkaloidal reagents, notably picric and tannic acids, previous acidification of the solution with hydrochloric acid is required for maximum protein precipitation. Phosphotungstic acid with the aid of hydrochloric acid is an especially effective protein precipitant; so that it may be used, with certainty, to render a solution protein-free. A number of other protein precipitants may be used for that purpose under properly controlled conditions of acidity and concentration. Some of the protein precipitation reactions have their applications outside the laboratory. The use of eggs, which are rich in protein, as an antidote for lead, copper or mercury poisoning, is due to the fact that proteins, if taken shortly after swallowing the metallic compounds, render them insoluble before they are absorbed from the stomach and intestine and, in this form, they may be safely removed by cathartics or stomach pump. The use of tannic acid or chromic acid in the hardening of hides, incidental to leather manufacture, is another application.

The *salting out* of proteins is useful in laboratory manipulations. All proteins, except the peptones, are completely precipitated, without chemical alteration, by saturating their solutions with solid ammonium sulphate. Several other salts, such as zinc sulphate, have the same effect. By similar use of sodium chloride, magnesium sulphate or certain other salts, only a few types of proteins may be precipitated. In acid solution, nearly all inorganic salts may be successfully used to salt out proteins. The different types of proteins vary as to their susceptibility to the salting-out process. Some, for example, may be thrown out by half saturation with ammonium sulphate, others by even one-third saturation, but the majority of proteins require saturation. Salting-out processes are obviously of value in separating various proteins from each other and the behavior of proteins towards salts is one of the criteria used in the practical classification and identification of proteins. There is no evidence to indicate strictly chemical combination between the protein and the neutral salts employed. Salting out is a physico-chemical process and is due primarily to the colloidal character of proteins.

The **heat coagulation** of proteins, though not strictly peculiar to them, is very useful in the detection and separation of many of them. The word coagulation is often used to signify any type of precipitation of colloids but many biochemists prefer to restrict the term to the precipitation by heat alone. Coagulation of a concentrated protein solution is typified by the hardening of egg white during boiling. In dilute solutions of coagulable proteins, such as the albumins and the globulins, a flocculent precipitate constitutes the coagulum. Different proteins tend to coagulate at different temperatures; some as low as 35°C., some as high as 70°C. but the process involved really begins at temperatures below that at which actual cloudiness and flocculation is seen and is merely greatly hastened at some critical temperature. The whole process is very sensitively influenced by the reaction of the solution. If the reaction is too strongly or too weakly acid no coagulation occurs and within the very narrow range of acidity permitting coagulation, any variation from the optimum greatly alters the temperature at which coagulation becomes visible and decreases the completeness of the separation of protein into a coagulum. At just the right degree of acidity, which may be experimentally determined for any given solution, all coagulable protein may be separated by boiling. In practice, the coagulation test is employed, as in the diagnostically valuable test for albumin in urine, by boiling after the addition of a small proportion of dilute (about 1 per cent) acetic acid.

Precipitation by alcohol of sufficient concentration is obtained with almost all proteins and after prolonged contact with alcohol the precipitate becomes insoluble in water, behaving like a heat-coagulated protein.

The Classification of Proteins.—Entirely satisfactory criteria for classification of such complex compounds as proteins are not available.

It would be an advantage to classify them strictly on the basis of their chemical constitution; but in the present state of incompleteness of protein chemistry, it is convenient to group them partly according to their constitution and partly according to their properties. Some proteins show behavior which places them on the border line between certain well-defined groups, so that different biochemists do not always agree as to complete classifications. The system adopted by the American Society of Biological Chemists, slightly modified to include some advantageous features of the system approved by the British Medical Association is given here.

Three main groups are distinguished:

- (A) Simple Proteins.
- (B) Compound Proteins.
- (C) Derived Proteins.

(A) THE SIMPLE PROTEINS.—Compounds occurring in nature and yielding on hydrolysis only α -amino-acids or their immediate derivatives.

1. *Albumins*, soluble in water and neutral salt solutions, coagulable by heat, salted out by saturation with ammonium sulphate but not by saturation with sodium chloride except in the presence of acid, give all protein color and precipitation tests and lack none of the known amino-acids among their hydrolysis products; examples: Ovalbumin from white of egg, serum albumin from blood plasma.

2. *Globulins*, insoluble in pure water and very dilute salt solutions, soluble in 1 per cent or slightly stronger solutions of neutral salts, coagulable by heat, salted out by half saturation with ammonium sulphate and by complete saturation with sodium chloride, give all protein color and precipitation tests and lack none of the known amino-acids among their decomposition products; examples: Ovoglobulin from white of egg, serum globulin from blood plasma, edestin from hemp seed, many globulins similar to edestin from other seeds and nuts, myosin from meat.

3. *Glutelins*, insoluble in water or neutral salt solutions but soluble in very dilute acids or alkalies, coagulable by heat, show no conspicuous lack of any amino-acid; examples: Glutenin from wheat, oryzenin from rice.

4. *Prolamines*, insoluble in all watery solutions but soluble in 60 to 80 per cent alcohol, not heat coagulable, contain the largest amounts of proline found in any proteins, amounting to 10 per cent or more of the molecule, also the largest amount of glutamic acid found in any proteins, amounting to 43 per cent in one case; examples: Gliadin from wheat, hordein from rye, zein from maize and others from various seeds.

5. *Albuminoids* or *Scleroproteins*, insoluble in all reagents which do not decompose them, digested slowly and with difficulty, if at all, by all gastro-intestinal enzymes, tend to yield a disproportionately large amount of simpler amino-acids such as glycocoll and are deficient or entirely

lacking in one or more of the more complex amino-acids such as tyrosine and tryptophane; examples: Keratin from epidermis, horns, hair, wool, nails and other skin appendages; collagen from bones, tendons and other connective tissues; fibroin of silk.

6. *Histones*, soluble in water and dilute acid solutions, insoluble in ammonia, soluble in sodium or potassium hydroxide, not coagulated by heat, predominatingly basic in character and yield comparatively large amounts of diamino-acids, occur in nature as components of compound proteins; examples: Globin of hemoglobin from blood, histones of nucleo-proteins from various plant and animal tissues.

7. *Protamines*, simple proteins of comparatively low molecular weight, soluble in water, dilute acids and alkalies (including ammonia) not coagulated by heat, so predominatingly basic that their watery solutions are alkaline to litmus, combine with large proportions of acid but have only a slight combining power for alkalies, composed largely of diamino-acids, especially arginine, occur in combination with nucleic acid in the heads of spermatozoa; examples: Salmin from salmon sperm, sturin from sturgeon sperm, clupein from herring sperm, several others that have been prepared from various kinds of fish sperm.

(B) THE COMPOUND PROTEINS.—Substances occurring in nature and yielding on hydrolysis, in addition to α -amino-acids, some non-protein group, sometimes called the prosthetic group.

1. *Nucleoproteins* yield nucleic acid, occur most abundantly in cell nuclei but not confined to them, are generally combinations of a histone with nucleic acid; examples: Thymus nucleoprotein, yeast nucleoprotein.

2. *Chromoproteins* or Hemoglobins, colored proteins, composed of a histone united to a color group containing a metal, include the respiratory pigment-proteins of blood; examples: Hemoglobin from blood, hemocyanin from invertebrate blood.

3. *Glycoproteins*, yield sugar-like substances as the prosthetic group; examples: Mucin from saliva, mucoid from connective tissues.

4. *Lecithoproteins*, yield lecithin (a phosphorized fat) as the prosthetic group, have not been sufficiently studied to make certain whether they actually occur in nature or are formed during the process of their preparation from various plant and animal substances, e.g., egg yolk.

5. *Lipoproteins*, yield fatty acid as the prosthetic group; like lecithoproteins, have not been definitely proved to occur in nature but may exist in all plant and animal tissues.

6. *Phosphoproteins*, sometimes classed as simple proteins since no organic prosthetic group has been identified; hydrolyze to yield phosphoric acid which has been regarded as the prosthetic group; are predominatingly acid in character because of the phosphate group and their high content of the dicarboxylic amino-acids (aspartic and glutamic);

important in nutrition of growing animals; examples: Casein from milk, ovovitellin from egg yolk.

(C) DERIVED PROTEINS.—Proteins obtained by partial hydrolysis of natural proteins. They are intermediary products of protein hydrolysis. The group also includes the slightly modified (probably not hydrolyzed) proteins obtained by heat coagulation and the synthetic substances called peptides.

1. *Coagulated proteins*, produced by heat or alcohol coagulation, which is apparently a process of dehydration; insoluble in all reagents which do not decompose them.

2. *Metaproteins* or *infraproteins*, produced by brief action of dilute acids or alkalies on natural proteins at temperatures below boiling; examples: Proteins formed by action of dilute acid on certain globulins at room temperature, alkali-metaprotein formed by action of dilute NaOH or KOH on natural proteins at 30 to 60°C., acid metaprotein formed by action of dilute acid on natural proteins at 30 to 60°C.

3. *Proteoses*, produced by action of dilute acids or protein-digesting enzymes when hydrolysis is permitted to go beyond the metaprotein stage; divided into primary and secondary proteoses; the primary proteoses are salted out by half saturation with ammonium sulphate and are precipitated by nitric acid and by picric acid, while secondary proteoses are salted out only by complete saturation with ammonium sulphate and are not precipitated by nitric or picric acid. The secondary proteoses have a smaller molecular weight than primary proteoses and represent a more advanced stage in the hydrolytic cleavage process of natural proteins. Proteoses are not coagulable by heat. Many of them are powerfully toxic when injected into animals. Example: Albumoses from albumin.

4. *Peptones*, produced by action of dilute acids or protein-digestive enzymes when hydrolysis is permitted to go beyond the proteose stage; can not be salted out by ammonium sulphate or any other salt; are not precipitated by nitric acid or picric acid, have a molecular weight small in comparison with natural proteins and have complexity and general chemical behavior very similar to that of artificially synthesized polypeptides which may, indeed, be defined as peptones of known molecular structure.

THE PHYSICAL CHEMISTRY OF THE PROTEINS

The Relations between Physiology and Physical Chemistry.—The border land between physics and chemistry has been brilliantly investigated during the past 35 or 40 years. So many of the discoveries in this field have an important bearing upon interpretations of the living mechanism, that all physico-chemical aspects of the behavior of any

biological substance are just as important as its molecular structure or other facts of a more strictly chemical nature.

The student should attempt to formulate some conception of the difference between physical, chemical and physico-chemical phenomena. The distinctions are not always easy to make and the classification of certain phenomena must be more or less arbitrary. It is clear that mere movement of matter without disruption of its molecular structure may be regarded as physical and that any atomic rearrangements involving the destruction of molecules and the formation of new ones is chemical. When, however, molecules are temporarily disrupted, as in electrolytic dissociation, or hold numbers of water molecules around them, as in the phenomenon of solvation which occurs in solutions, or aggregate with molecules of their own kind to produce the colloid state, the process may be conveniently termed a physico-chemical one.

Historically, physical chemistry and physiology have not been unrelated in their development and certainly physiology would have been greatly hampered in growth without a simultaneous or previous development of physical chemistry. One of the earliest important discoveries in physical chemistry was made by a botanist, Pfeffer, who first quantitatively investigated osmotic pressures and thus enabled Van't Hoff to formulate the law of osmotic pressure; while the work of DeVries on plasmolysis in plant cells was of fundamental importance to Arrhenius in reaching his theory of electrolytic dissociation. (See Chap. VI.)

An outstanding feature of the physical chemistry of the proteins is their colloidal behavior. Proteins are *colloids*. The word (from the Greek for glue) was given by Graham (1861) since glue and similar substances seemed typical of the class. The chief constituent of ordinary glue is gelatin, a protein. Graham's work laid the foundation of colloidal chemistry.

The Nature of Colloids.—For an understanding of the nature of colloids, a conception of the size of the individual particles, of which some consist, is helpful. Imagine a cube of gold of 1 mm. edge, obviously a solid body of macroscopic size, to be divided into a billion equal pieces of 1μ edge. The resulting particles, though now microscopic in size, would still be typical solids and if suspended in water would quickly sink as a fine gold dust; but if each of these particles were in turn divided into a million equal pieces of 0.01μ ($10\mu\mu$) edge, each of the resulting fragments would be beyond the range of visibility by use of the highest powers, even with oil immersion objectives, of the ordinary microscope. The smallest objects that may be seen microscopically have a diameter of approximately $200\mu\mu$, that is, one half the shortest violet rays in the visible spectrum—the practical limit of visibility. Such fine particles of gold, if they could be uniformly dispersed in water, would remain suspended, under certain circumstances, and would give a clear, transparent and

fairly stable solution. Yet the gold would not be in *true* solution since its particles would still be so much larger than those of gold atoms or ions. It would be in colloidal solution. Such solutions would show a ruby red, violet or blue color whereas the gold ion seen in true solutions of gold salts, such as the chloride, are red. There is other evidence sufficient to show that gold in such a state of subdivision is not in true solution but in what is called the *colloidal state*.

Of course we have no practical means of procuring a colloidal gold solution by simple mechanical subdivision of the solid; but the reverse process, by which gold ions in solution are aggregated into particles of colloidal size, may be effected in several ways. Faraday (1858) first showed that solutions of gold chloride treated with reducing agents gave beautiful red or purple solutions of gold. A number of precautions are important for the preparation of such solutions. Freedom from impurities is especially necessary. Dust from the air, traces of silicates dissolved from the flask if not made of highly insoluble glass, or a slight excess of the reducing agent will cause a precipitation of solid particles of metallic gold which slowly form by aggregation of the ultramicroscopic particles into microscopic ones which are too large to remain suspended in the water. In other words, the stability of a colloidal solution is dependent upon the existence of particles of sizes which vary only within certain limits.

Exact definition of these limits cannot be given because all gradations of size of particles exist in solutions. They vary from the minute hydrogen ion and other small ions and molecules to the comparatively large aggregates of starch molecules in the colloidal suspension called starch paste and the big aggregates of cloudy protein solutions. The size of particle, capable of remaining in the colloidal state, varies under different circumstances. It depends on the nature of the substance in the colloidal solution especially its specific gravity in relation to that of the solvent and on the character of the solvent itself. Every gradation between a true solution, with the dissolved substance in a state of molecular or ionic subdivision, and the typical colloidal solution is to be found. Mere subdivision into molecules or ions is not necessarily a distinguishing feature of a non-colloidal solution since many kinds of protein molecules or ions, as well as starch and a number of other organic molecules are so large that they can exist only in the colloidal state. Such substances may be regarded as typical colloids.

It was supposed at the time of Graham's work that all chemical compounds might be divided into colloids and crystalloids. We now realize that such a classification is misleading. Many typical colloids can be crystallized, for example, proteins. There is a possibility that under suitable conditions every so-called crystalloid may be put into the colloidal state. Various ones have been so manipulated. The list includes: Gold, silver, platinum, copper and iron among the metals, many metallic

hydroxides, organic acids, and a number of organic and inorganic salts. Even so typical a crystalloid as sodium chloride has been put in the colloidal state. The word colloid, then, is not to be regarded as referring to the chemical constitution of substances except in the case of those which, like most of the proteins, can exist only in large aggregates because the molecule itself is very large.

Types of Colloids and Their Occurrence.—The colloidal state is not confined to substances in liquid solutions. Any finely divided matter suspended in any medium constitutes a colloid. Much of the material dealt with in our every day life, in the arts and industries, is in the colloidal state. This is clearly realized upon examination of a list of the different kinds of colloids together with some examples of each type. Every sort must consist of two dissimilar materials, the one finely subdivided and suspended in the other. These two are called phases: (1) The *dispersed* (divided) or *internal* phase, and (2) the *continuous* or *external* phase. Either phase may be gas, liquid or solid. Both phases may be liquid or solid. Both phases cannot be gaseous because of the freedom of diffusion of gas molecules.

TYPES OF COLLOIDS	EXAMPLES
1. Gas dispersed in a liquid.	Foam.
2. Gas dispersed in a solid.	Floating soap.
3. Liquid dispersed in a gas.	Clouds and mist.
4. Liquid dispersed in another immiscible liquid.	{ Emulsoids, like skim-milk. Emulsions, like cream.
5. Liquid dispersed in a solid.	Jellies, like ordinary gelatin.
6. Solid dispersed in a gas.	Smoke and dust.
7. Solid dispersed in a liquid.	Colloidal solutions of gold, arsenious sulphide, etc., suspension of coagulated albumin.
8. Solid dispersed in a solid.	Certain types of stained glass.

It is clear that colloidal materials are common enough. One author has spoken of them as "bubbles, drops and grains." All protoplasm is in the form of colloidal solution. Many biological fluids, notably blood, lymph, milk, bile and digestive secretions are colloidal solutions. The colloids that are met with chiefly in physiology are of types 4, 5, and 7: Liquid dispersed in another immiscible liquid (including most protoplasmic and biological fluids), liquid dispersed in a solid or semisolid (including protoplasmic and other jellies) and solids dispersed in a liquid (including various colloidal solutions of biological interest). Colloids dispersed in liquids, types 4 and 7, are sometimes spoken of respectively as **hydrophil** and **hydrophobe**.¹ The hydrophil colloids appear to attract water and are easily dissolved while the hydrophobe group do not appear to attract water and are put into colloidal solution only by special manipulations

¹ If solvents other than water are used to disperse them they may be termed lyophil and lyophobe.

like the reduction of a true solution of gold salt or coagulation of a protein solution. The terms reversible colloid, meaning capable of being redissolved, and irreversible, not to be redissolved, are used as distinguishing names and in most cases reversible is synonymous with hydrophil and irreversible with hydrophobe. But newer knowledge of the colloidal state has made it apparent that all hydrophil colloids behave like liquid droplets surrounded by the solvent, in other words like an emulsion, while the hydrophobes have all the characteristics of minute specks of solid matter suspended in the dispersing medium. On this account substances in colloidal solution are now quite generally classified as **emulsoids** and **suspensoids**. It is difficult to conceive of a protein solution as a series of ultramicroscopically small protein solutions each surrounded by water. We can only account for it by supposing that the proteins possess cohesive forces which hold a certain number of molecules of water in physico-chemical combination within or around each colloidal protein particle.

Detection of Colloids by Optical Means.—Inasmuch as the colloidal state is merely a matter of comparative size of aggregates, some practical

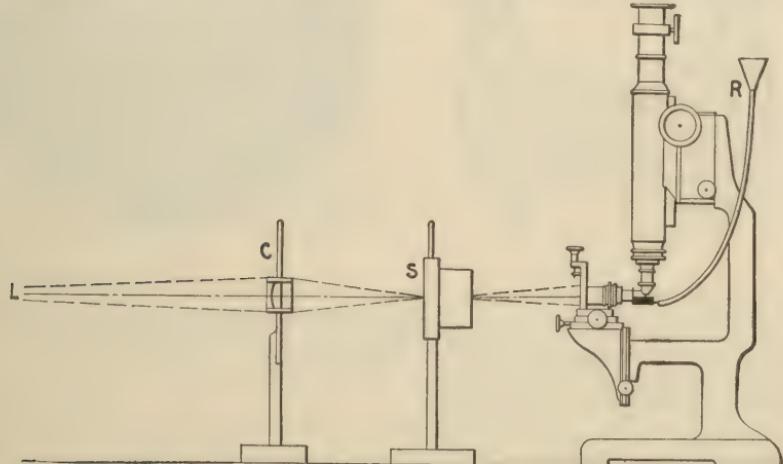


FIG. 9.—Diagram to show arrangement of ultramicroscope. *L*, source of bright light such as an arc lamp. *C*, a condenser. *S*, narrow slit to admit a pencil of light rays of definite size. *R*, reservoir to supply colloidal solution to be examined.

and more or less arbitrary measurements must suffice for its detection. The most striking phenomenon used in detection of the colloidal state is the Faraday-Tyndall effect. It is well illustrated by the motes in a beam of sunlight. A bright ray shining through a comparatively dark room renders the particles of dust in its path clearly visible although the air of the room when uniformly illuminated seems perfectly clear and transparent. Similarly a beam of brilliant light passing through a colloidal solution shows its path marked out by a cloudy haze resembling motes in a sunbeam. A simple experiment will illustrate. A lens in front of

an electric or other bright light in a dark room is arranged to throw a pencil of light into a solution of gum mastic in alcohol. Only a few flecks will be in its path, no more than are visible in pure alcohol or distilled water. They are due to small amounts of impurities such as dust in the liquid. Mastic in alcohol is in true solution and does not affect the light. If a few cubic centimeters of alcoholic mastic solution are then poured into about a liter of water a slightly milky or opalescent solution appears and this, traversed by the beam of light, shows a bright cloud of particles in its path because mastic aggregates into colloidal particles in water. The Faraday-Tyndall effect is utilized in the *ultramicroscope*. The arrangement of lamp, lenses and microscope is shown in Fig. 9. With-

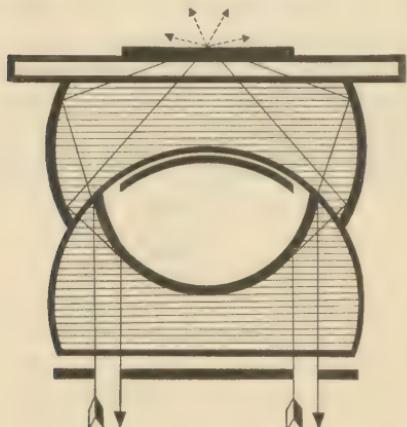


FIG. 10.



FIG. 11.

FIG. 10.—Vertical section of the reflector used in the sub-stage of a microscope to furnish dark field illumination. The path of the light rays is indicated by arrows. By this arrangement the material on the microscope slide is illuminated from the side and no light is thrown directly into the objective of the microscope.

FIG. 11.—Field seen in the ultramicroscope. (After Bayliss, "Principles of General Physiology," Longmans, Green & Co.).

out going into details of construction, which may be found in works on colloids, suffice it to say that bright, concentrated light is focused by the lenses of a compound microscope at the center of the field of another high power microscope with its optical axis at right angles to that of the one used to focus the light. Other light is excluded from the field. The dark field illumination apparatus employs a similar principle. It is illustrated in Fig. 10. The Faraday-Tyndall phenomenon renders particles in the solution visible. What is the cause of the phenomenon? If one remembers the length of visible light waves (approximately $400\text{-}700\mu\mu$) it is clear that an object to appear in anything like its true shape and size must be large enough to actually reflect light waves. Anything with a diameter less than half the length of the light waves falling upon it does not sufficiently stop or reflect light to permit the formation of a true image.

Smaller objects, however, do affect the light waves to produce the phenomenon called diffraction. It may be imagined as a sort of scattering of the light. The object causing diffraction appears as a hazy spot of light much larger in its apparent size than the actual object. The appearance of a colloidal solution in the ultramicroscope is shown in Fig. 11. It is practically impossible to prepare a liquid of such purity that it presents a lightless, optical void in the field of the ultramicroscope. Minute particles of dust are visible in pure solvents or true solutions; but when a colloidal solution is observed, the cloud of light points or the milky haze of countless points, too minute to be separately distinguished, presents an appearance distinctly characteristic of the colloidal state. Of course many colloids look distinctly milky or translucent to naked eye vision—milk itself, for example, or cream in which the colloidal particles are droplets of fat in emulsion, or skimmed milk whose bluish opalescence is due to colloidal particles of the protein, caseinogen. Such solutions possess colloidal particles of comparatively large size. But many solutions, quite transparent to the unaided eye, are obviously colloidal in the ultramicroscope. This is the case with the majority of protein solutions so that the ultramicroscope becomes a useful index of the colloidal state. It is convenient to classify roughly the various sizes of particles which may be distinguished. The term **microns** is used to refer to small objects that are large enough to permit formation of definite, focused images. Objects, too small to form images but distinguished in the ultramicroscope as distinct disks of light, are called **submicrons**; while those, visible only as an indistinct haze even in the ultramicroscope, are called **amicrons**.

The actual mass of submicrons has been estimated by an ingenious method. The number of colloidal particles in a measureable microscopic volume of a very dilute suspension is actually counted in the field of the ultramicroscope. The concentration of the colloid in the solution is then obtained by quantitative analysis; so that division of the amount of substance, in a given volume, by the number of particles observed permits an estimation of the average mass of individual particles. If the particles consist of something of known density, as in the case of colloidal gold solutions, their size may be computed. In this way it has been shown that particles of gold as small as $1.7\mu\mu$ ($\frac{1.7}{1,000,000}$ mm.) in diameter were actually recognized, under favorable conditions, as submicrons.

This is of the same order as the dimensions of the largest molecules. Starch, for example, has been given an estimated molecular diameter of $5\mu\mu$. Still, we have no satisfactory evidence that any single molecule, even one of starch or of protein, is visible as a submicron. This is due largely to the fact that small particles of these substances have a refracting power very nearly equal to that of the surrounding liquid, so that they

do not disperse light to the same extent that gold particles do. The protein particles that have been seen as submicrons are regarded, somewhat arbitrarily, as aggregates of molecules. Individual protein molecules, as commonly defined in chemistry, have not been seen even in the ultramicroscope.

Detection of Colloids by Their Non-diffusibility.—The use of dialyzing membranes in the study and detection of colloids has been of interest since the days of Graham. The membranes employed are semipermeable, that is, permeable to some molecules but impermeable to others. One permeable to water but not to anything soluble in water has never been produced although membranes impermeable to many solutes have

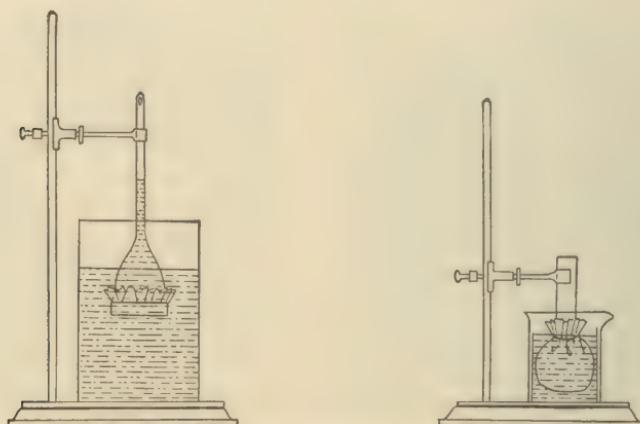


FIG. 12.—Apparatus used for dialysis.

been prepared. Vegetable parchment has been used most extensively and was introduced by Graham. Collodion membranes are also useful and for exact quantitative work the precipitation membrane of copper ferrocyanide has been found best. The latter is formed in the walls of a cup of porous clay (used as a support for the membrane) when copper sulphate solution within the cup diffuses into the walls to meet potassium ferrocyanide solution diffusing from without. With any of these three kinds of membranes various degrees of semipermeability may be obtained by preparations of varying thickness and density.

When the membrane is arranged to test the ability of a solute to diffuse through it, the whole arrangement is called a **dialyzer** and the process **dialysis**. Various types of dialyzers are shown in Fig. 12. The solution containing the substance to be tested is placed on one side of the membrane and water on the other. If, after a suitable time, the solute may be found in the water and if the membrane is truly semipermeable so as to prevent any filtration, it is certain that the solute crossed the membrane by diffusion. Different substances known to be of different molecular weight

have been tested in dialyzers with membranes so made as to vary in density and presumably, therefore, in the size of the pores or openings in their meshes. It was found that the more dense and compact the membrane, the smaller were the molecules of substances which it could prevent from dialyzing. Through any given membrane, then, all molecules or ions or colloidal particles above a certain size cannot diffuse while smaller bodies can. This is equivalent to saying that the membrane is a sieve with holes not exceeding a certain diameter. It is true that in many cases the rate of diffusion is markedly affected by the electrical condition of the membrane, that is, whether it is electrically neutral or has acquired a positive or negative charge. But this, while affecting the rate, does not determine whether or not a given substance can pass through the membrane. Dialysis of some substances through some membranes is due to solution of the substance in the membrane itself. This is the explanation of the passage of hydrogen gas through the metal palladium which dissolves hydrogen. This sort of dialysis has been postulated for living membranes but has not been proved to occur. In the main, the sieve theory agrees with observations on biological materials, so that dialysis is a practical test, the simplest and more readily available, for the detection of the colloidal state.

As a somewhat rough criterion, it is pretty generally agreed that *any substance which diffuses through ordinary vegetable parchment, is not colloidal. Substances held back by this membrane, are called colloidal.* Proteins are nondiffusible. They are colloids. The only exceptions to this rule are the peptones, which are slowly diffusible, and some of the proteoses, which are still more slowly diffusible.

Dialysis of protein solutions against frequent changes of distilled water is a method of separating proteins from diffusible substances such as inorganic salts. It is widely employed. In the case of globulins, which are soluble in salt solutions but insoluble in water, a good method of preparation and purification is to dialyze the globulin solution against distilled water until salts have diffused out sufficiently to cause precipitation of the globulin within the dialyzer.

Separation of Colloids by Ultrafiltration.—Another application of semipermeable membranes to colloidal chemistry is ultrafiltration. Through all ordinary filters, regarded as "permeable," colloidal solutions pass more or less freely. This is familiar in the case of milk which, though filtering slowly and tending to clog filter papers with fat droplets, can be filtered. Skimmed milk, fat-free, is easily filterable. The passage of sulphur and sulphides through paper, when they produce a colloidal solution during precipitation of metallic sulphides, is another example which will hardly be forgotten by students of analytical chemistry who have encountered the difficulty. Protein solutions are in general filterable although, like all colloidal solutions, with some difficulty.

The ultrafilter, unlike ordinary filters, is composed of a semipermeable membrane of gelatin or collodion deposited upon a porous cup strong enough to withstand high pressure. It is capable of holding back colloids and letting through other constituents of a solution, when filtration is conducted under a pressure of several atmospheres.

Colloidal particles may also be separated from a solution by centrifugal force. In a centrifuge of comparatively low speed, less than 5,000 revolutions per minute, the larger and heavier of colloidal particles may be thrown out; while in a very powerful centrifuge, at 10,000 or more revolutions per minute, colloids in much higher states of dispersion have been separated.

Behavior of Proteins toward Polarized Light.—All proteins exhibit optical activity which means that they affect the plane of polarized light. Ordinary light may be pictured as electromagnetic vibrations pulsating in many planes. When a ray of ordinary light passes through a crystal of Iceland spar, a form of calcite, it emerges as two rays. The light has been "sorted" into two kinds. One ray behaves like ordinary light, but the other behaves as though its vibrations were all in one plane and is therefore said to be plane polarized. This is recognized by the observation that such light can traverse a second crystal of Iceland spar only when it is in certain definite positions relative to the first crystal; namely, with its faces in a relation to the path of the polarized ray corresponding to that taken by the first crystal. A number of substances besides Iceland spar cause polarization of light, but it is most generally used for the purpose. Very many organic substances affect polarized light by giving a twist or *rotation* to it and since some substances rotate the plane to the left and some to the right with a power, capable of quantitative measurement and characteristic for each substance, the so-called *optical activity* of organic compounds is very useful in their identification and quantitative determination.

The instrument for production and observation of polarized light is called a *polariscope*. The instrument is shown in Fig. 13. The polariscope contains two Nicol prisms. The Nicol prism, named after its inventor, is constructed of two pieces of Iceland spar so fitted together that only the polarized ray of light emerges while the ordinary ray is refracted away from the optical axis of the prism. The polariscope may be considered to be, essentially: (1) A polarizer, containing a Nicol prism to which is admitted light from a suitable source and from which emerges only polarized light, (2) an analyzer, containing a Nicol prism or prisms for the detection of the plane of the polarized light, and (3) a small telescope for observing it. Between the polarizer and analyzer is a chamber in which solutions to be observed may be placed. If the polarizer and analyzer are set, by rotation on its optical axis of one or the other, so that polarized light passes through the analyzer

unobstructed, an observer looking into the eyepiece of the telescope sees no shadows in its field. If now a solution of an optically active substance is placed in a tube in the chamber between polarizer and analyzer, shadows will appear in the field. By rotating either polarizer or analyzer through a sufficient angle, the clear field again appears in the telescope. Rotation has compensated for the rotatory power of the optically active substance.

If the concentration of it in the solution and the length of the tube through which the light passes are known, then, from the amount of rotation required to compensate, the comparative intensity of the optical activity of the substance can be computed. The resulting figure is

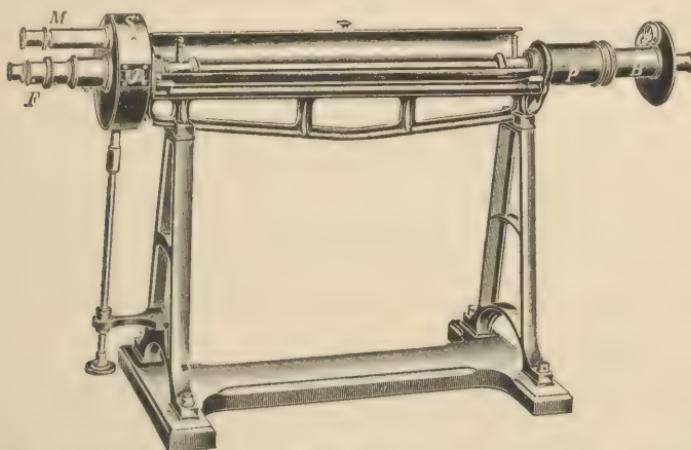


FIG. 13.—Polariscope. *P*, polarizer. *B*, chamber into which light filters may be introduced. *A*, analyzer which rotates within the circular chamber and is turned by the screw with milled head. The scale is read through the small telescope opposite *S*. The telescope below this is for observation of the polarized light. (*From the catalogue of Eimer & Amend*).

known as the **specific rotation** of the active substance. In the case of some substances, the temperature of the solution markedly affects its rotatory power so that temperature must be taken into consideration in making the computation. In most cases, the effects of the variations which would occur in the temperature of the laboratory are smaller than the effects of experimental error in determination of the amount of rotation; so that temperature may be neglected. The kind of light used is also important since light waves of different length are differently affected. This variable is eliminated by the use of monochromatic light, that is, light of uniform wave length. It is obtained by heating a sodium salt, usually the carbonate, in a colorless flame. This gives the familiar sodium flame with light of practically one wave length and since the waves appear in the spectrum at the location of the D line of the solar spectrum, light from the sodium flame is called D light. Since it is used, by common consent, in polaroscopic observations, computations for specific rotation

do not need to take into account the effects of quality of light. The amount of rotation actually observed is read in angular degrees from a circular scale on a disk attached to and rotating with the polarizer or the analyzer—according to which one is attached, in any particular instrument, to a rotating device. The scale may be read through its own telescope.

In practice, the observer rotates until polarizer and analyzer are so matched as to give a clear field in the telescope and takes the reading of the scale. He then introduces the solution, again rotates to obtain a clear field and observes the reading. The difference between the two readings is the angle of actual observed rotation. Its amount depends on the specific rotation of the optically active substance, its concentration in the solution and the length of the tube. The relation is given in the following equation:

$$\alpha = (\alpha)_D \times l \times W,$$

where α = observed angle of rotation in degrees,

l = length of the tube in decimeters,

W = weight of the substance in grams in 1 cc. of the solution,

$(\alpha)_D$ = specific rotation of the substance observed in sodium light.

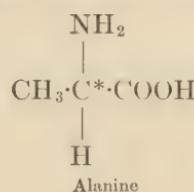
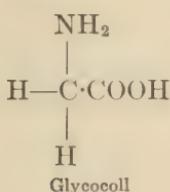
Since l and W may be chosen at will and α may be measured in the polariscope, $(\alpha)_D$ may be computed. For this purpose, the above equation is used in the form: $(\alpha)_D = \frac{\alpha}{l \times W}$.

If the tube were 1 dem. long and the solution contained 1 gram of material in 1 cc. of water, $l \times W$ would equal 1 and $(\alpha)_D = \alpha$; so that specific rotation of a substance may be defined as *the rotation of polarized sodium light, expressed in angular degrees, obtained when one gram of substance is dissolved in 1 cc. of solution and observed in a tube 1 dcm. long.*

Actual observations are, of course, impossible with 1 gram dissolved in 1 cc. of water or any other solvent since organic substances do not possess that degree of solubility. Measurements made at a convenient concentration are reduced to standard conditions. For example, a 5 per cent solution of glucose in a tube 2 dem. long, showing a rotation of 5.25° , would have a specific rotation of 52.5. If, by noting the direction in which the instrument must be rotated to obtain compensation, it is seen that the substance turns the plane of polarized light clockwise (observer facing the telescope) the substance is called *dextrorotatory* signifying that it turns to the right. Counterclockwise rotation is termed *levorotatory*. These signs + and - are used, respectively, as abbreviated expressions of dextro- and levorotation. Thus glucose, rotating to the right, is known as dextrose and its specific rotation is given as: $(\alpha)_D = +52.5$.

Asymmetry as the Cause of Optical Activity.—All proteins are optically active and their constituent amino-acids, with the exception of glyco-

coll, are likewise. What is the cause of optical activity? From many observations on substances of known molecular structure, it has been clearly demonstrated that arrangement of atoms is the cause. Any organic compound in which one or more of the carbon atoms is attached to four different kinds of atoms or groups is optically active. Such a carbon atom is called *asymmetric*. Compounds lacking an asymmetric carbon are inactive. Thus glycocoll shows no rotation; while alanine,



whose asymmetric carbon is indicated by an asterisk, is optically active. Examination of the formulas for amino-acids as shown on pp. 25-27 will make it clear that they are all, except glycocoll, asymmetric. The majority of them are levo-, the others dextrorotatory, and since they are built bodily into protein molecules it is to be expected that proteins would be optically active.

It is one of the most startling facts of biochemistry that, while every asymmetric compound found in nature is optically active, many of the same things, produced by strictly artificial synthesis, are quite inactive. This very puzzling observation received its first explanation in some work of Pasteur¹ on tartaric acid. By careful microscopic observation of crystals of an inactive preparation, he found it to consist of seemingly equal numbers of two types of crystals, one so formed as to be the mirror image of the other. After sorting the crystals by actually picking out each kind under the microscope, he found that one kind was dextrorotatory while the other had exactly the same optical action in the levo direction. The inactive preparation really consisted of a mixture of equal parts of oppositely active ones. Such a mixture is called *racemic*. If the dextrorotatory type is written d-tartaric acid and the levo one l-tartaric acid the racemic (r-tartaric) could be written d-l-tartaric. Two substances of the same constitution, but differing from each other in that they show equal rotatory power in opposite directions, are called *optical isomers*. Since the only difference between them is the special arrangement of their constituent groups they are also called stereoisomers (from the Greek for space).

The study of special arrangement of atoms within molecules is known as *Stereochemistry*. Polaroscopic observations constitute one of its chief methods of investigation. It has developed the proportions of a

¹ Although Pasteur is usually thought of as the founder of Bacteriology and Immunology, his work in Physiology and Chemistry was also fundamental.

distinct branch of science with special treatises and an extensive literature of its own.

Theoretically, every optically active substance, possessed of one or more asymmetric carbon atoms, could have an optical mate. The reason for this has received an explanation partly based on Pasteur's discovery. Through the work of Le Bel and others, it has been shown that just as each of the two kinds of crystals of tartaric acid was the mirror image of the other, so an asymmetric carbon atom and attached groups may have its mirror image. Such a carbon atom, conceived as a tetrahedron with four valences at its four corners and attached to four different groups, is represented in Fig. 14. If alongside it is seen its mirror image, one notices how the four attached groups appear in reversed order and if to

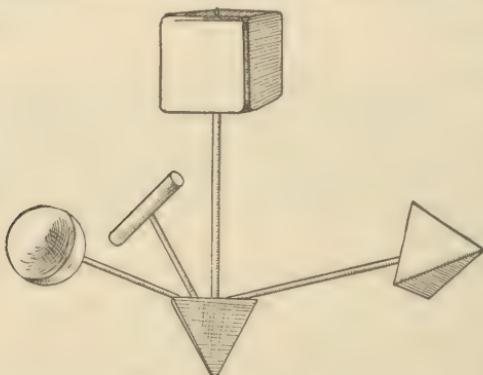
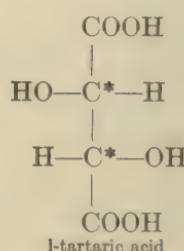
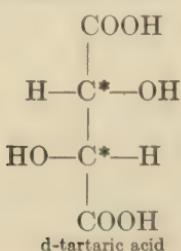


FIG. 14.—Diagram to suggest an asymmetric carbon atom. (After Bayliss).

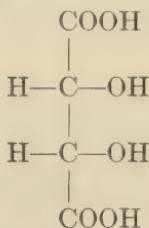
a model of a carbon atom, one attaches four different objects and then to a second model attaches the same four kinds of different objects, but in reverse order as pictured in a mirror image, he will now find that no turning of the first model can bring it into a position coincident with or superimposable upon the second; so that with the same combination of four different things attached to carbon, the result is not the same in the two cases. One of a pair of optical isomers, then, has the groups about its asymmetric carbon in reversed order as compared to that occurring about the corresponding carbon of its twin. The molecule of the dextro type is the mirror image of that of the levo type. For tartaric acids this fact may be represented thus:



A better representation would be in three dimensions. Confined, by the necessities of printing, to two dimensions it looks as though, by lifting one of them and turning it face downward, it could be superimposed upon the other. A three dimensional model shows that they are really not identical.

The racemic acid could be represented only as a mixture of equal parts of the two shown. In artificial syntheses of organic substances, it is the racemic form which almost invariably results. It would seem as though mere chance, something too variable to be explained, decided whether the d-form or the l-form should be produced. Difficult and laborious indeed are some of the processes required to get a separation of the "twins" and so obtain a substance, pure according to polaroscopic test. Nature, on the other hand, in all her synthetic processes, never forms a racemic compound but always one and one only of the seemingly possible optical isomeres. Amino-acids as obtained from proteins are always characteristically active; while only by very special types of synthesis, can artificial amino-acids, other than racemic, be formed.

The situation is still further complicated in that compounds may become inactive through an internal compensation within the molecule. The arrangement about one carbon atom may turn polarized light to the right, while an equal twist to the left is given by the group around the adjacent carbon. The net result is no detectable effect on the light. Such an arrangement exists in mesotartaric acid, represented thus:



By comparison with d-tartaric and l-tartaric one can see the explanation of its inactivity. Intramolecular compensations of this sort do occur in natural compounds but, as a rule, do not cause complete inactivity because in large and complex molecules the numerous asymmetric carbon atoms causing a twist to the right are not exactly balanced by those of opposite effect.

Biochemical Significance of Asymmetry.—The fact that all asymmetric, natural, organic compounds are optically active and never racemic takes on great interest and significance in the light of such observations as the following: (1) Bacteria, moulds and fungi utilize, in fermentation or other biological activities, one of the pair of optical isomeres in a racemic compound leaving the other untouched until the favorite is used up, when they may, at a slower rate, make some use of the other

form. (2) Enzymes of the secretions and tissues of plants and animals are constructed, in general, to act much more readily on naturally occurring compounds than upon their optical mates. (3) Again, only a certain optical type of amino-acid is ever used to build up proteins in the animal body. The opposite type may be destroyed so as to yield some energy value but never appears to be utilized as bricks for vital architecture. (4) Likewise, among sugars only a certain type of optical structure enables them to be of full usefulness to life. A number of other similar facts might be cited; so that it is clear that living things are extremely "particular" concerning the structure of the carbon compounds which they produce by synthesis or which they "select" for food purposes. The specifications cover not only the atomic grouping in the molecule but even the arrangement of each group about each carbon atom.

Upon such facts as these, much interesting biological theory has been based. It has been suggested that living matter from its earliest existence upon the earth has always been composed, so far as its carbon compounds are concerned, of optically active material. Since enzymes, present in such protoplasm, would have to be constructed of optically active material, in so far as they are carbonaceous, they might be especially adapted to affect only optically active substances. Enzymes are the chief reagents of living cells (see Chap. IX) so that most, perhaps all, vital activities are dependent upon the nature of the action of cellular enzymes. It follows that living matter utilizes only the especially adapted forms of optically active material, is itself composed of highly specific stereo compounds and produces only optically active compounds. As Reichert has pointed out, we have in stereoisomerism an aid to our conception of how heredity may be specific to the degree that we wonderfully observe. "Like begets like" within the comparatively narrow limits of natural variability and mutation. The near perfection of the resemblance between offspring and parent could only be possible with a highly specific physico-chemical composition of protoplasm. Mutations, which appear to break the continuity of inheritance, may involve stereochemical rearrangements, though concrete evidence is lacking.

The Physiological Role of Proteins.—Proteins are preeminent among the organic compounds that confer specific individuality to living structures. Not only are the proteins of any given species of plant or animal peculiar to it, but each protein from each kind of tissue is absolutely unique—not to be found elsewhere in nature.

This protein individuality is well illustrated by experiments in blood transfusion. The blood of one species of animal can not be transfused into the circulation of an animal of another species without disastrous results. Even the injection of a small quantity of foreign blood may be fatal. For example, the injection of 5 cc. of human blood into an average sized rabbit produces toxic effects and is often fatal. This toxicity is due to

the proteins of the foreign blood, because the fatal effect, preceeded by identical symptoms, is obtained after injection of isolated, purified protein prepared from the foreign blood or, indeed, from any source other than the body of a closely related animal. Because foreign proteins are incompatible with life, transfusions of the blood of any of the domestic animals into human beings are fatal; but the blood of certain anthropoid apes or human blood may be thus administered with only slightly disturbing symptoms.

Even the proteins from corresponding portions of different individuals of the same species are not necessarily identical. The blood proteins of one person, for example, are not always identical with those of another; so that in modern surgical practice of blood transfusion, the donor of the blood and the recipient are both given a preliminary blood examination in order to determine whether or not their bloods are compatible. Transfusion of an incompatible blood causes dangerous effects, such as agglutination (clumping together) of corpuscles, in the recipient.

Carbohydrate materials are not so varied in nature as proteins. In the entire animal kingdom, for example, only two carbohydrates can be shown to exist, as such, actually free in body tissues. They are dextrose and glycogen. A very few other sugar-like substances are in combined form in animal structures but their number is much less than that of proteins.

With fats and fat-like bodies one is struck with the similarity of the representatives in the various tissues of various species much more than with the limited variations.

That the protein should be able to confer peculiar chemical composition to any number of living structures is not surprising when we remember that, so far as we can see, any number of molecules of each or all of some eighteen or twenty amino-acids may be joined in any possible order. The permutations and combinations, therefore, are practically infinite. Biochemical individuality of species, of single individuals, of organs, of tissues, of processes is made possible by the complexity of protein structure.

The proteins as amphoteric colloids take an important place in life processes. Since they combine with acids and bases they can neutralize any excess of either in protoplasm. Although that object is largely attained by other means, proteins, if required to do so, can check the accumulation of H⁺ or OH⁻ ions¹ over a considerable range.

Since proteins are colloids they do not diffuse through semipermeable membranes such as form the boundaries of living cells and the walls of

¹ The student is presumably familiar with the general conception of ions as electrically charged particles occurring generally in solutions. It is important to recall that acidity and alkalinity are respectively a preponderance of H⁺ ions over OH⁻ and of OH⁻ over H⁺ while neutrality means an equal concentration of both.

other tissue structures in plants and animals. For this reason, proteins and many substances which may combine with them in a *non-diffusible form* can be retained in the individual cells.

The proteins have a *high chemical potential* which is another way of saying that they readily react with very many substances. Recalling the —NH₂ groups which can effect dissociation in the presence of water to form —OH' ions and the —COOH groups which can dissociate to form H' ions, one readily appreciates why they should combine with a certain amount of either acids or bases. But in experiments on several different proteins it has been shown that they combine with a much larger proportion of acid than could possibly be taken up by their —NH₂ groups and, on the other hand, they combine with more alkali than can be accounted for by the —COOH groups of the protein molecule. An entirely satisfactory explanation of this fact has not yet been given. For experimental evidence and theoretical considerations, the reader is referred to Robertson, *The Physical Chemistry of the Proteins*. Whatever the final explanation may be, the fact itself emphasizes the most significant property of proteins, namely, their great reactivity.

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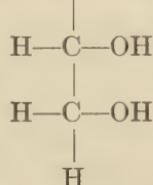
CHAPTER III

THE TRUE FATS, THE LIPOIDS AND RELATED SUBSTANCES; THEIR CHEMISTRY AND THEIR RÔLE IN LIFE PROCESSES

Although less abundant than proteins and less prominent in their effects upon nutrition, yet the group of substances known as fats, like the proteins, are widely distributed in living structures and appear to be present, at least in some modification, in all living things. The group includes the true fats, the lipoids or fat-like compounds and the sterols. The latter differ from the fats in chemical structure but have certain properties which relate them to the fats. It is convenient to describe these three types of substances separately.

Definition of the True Fats.—The true fats, sometimes called neutral fats, are the triglycerides of the fatty acids. The term, triglyceride, refers to the fact that the glycerol (glycerine) radical is part of the molecule and is combined with three radicles from fatty acid groups. Glycerol is an alcohol. All alcohols contain the $-\text{C}-\text{OH}$ grouping as, for example, in methyl alcohol, CH_3OH and ethyl alcohol, $\text{C}_2\text{H}_5\text{OH}$; but the higher alcohols contain more than one $-\text{C}-\text{OH}$ group. Glycerol

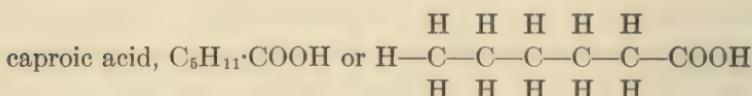
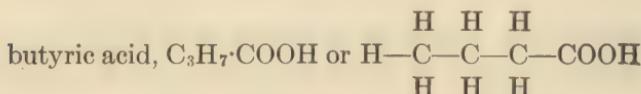
contains three of them. Its structure is: $\begin{array}{c} \text{H} \\ | \\ \text{H}-\text{C}-\text{OH} \end{array}$, also written



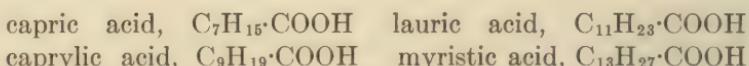
$\text{CH}_2\text{OH}\cdot\text{CHOH}\cdot\text{CH}_2\text{OH}$, or $\text{C}_3\text{H}_5(\text{OH})_3$. In any alcohol, the $-\text{OH}$ radical is replacable by others, especially by acid radicals and since glycerol can thus incorporate three acid radicals, it is known as a triacid alcohol. The combination of an alcohol with an acid is called an ester. As fats are such combinations they are sometimes called triglyceride esters.

The Fatty Acids.—Very many different fatty acids are known; but of this large number, only a few are ever found in combination with glycerol as natural fats. Of the so-called normal fatty acids, for example,

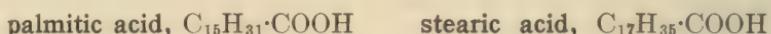
there is a long series whose general formula is $C_nH_{2n+1}COOH$. Specific members of the series are: Acetic, $CH_3\cdot COOH$, propionic, $C_2H_5\cdot COOH$, butyric, $C_3H_7\cdot COOH$, etc. Of this long series, however, only the members that contain an even number of carbon atoms have ever been found in either plant or animal fats. They include:



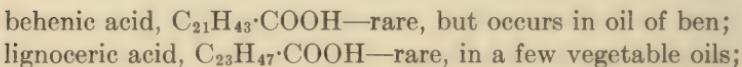
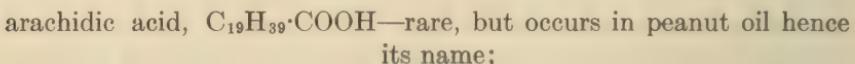
rare in most fats but compose 5 per cent of the fatty acids in butter;



very scarce or lacking in animal fats, found in vegetable fats, rarely in large amount;

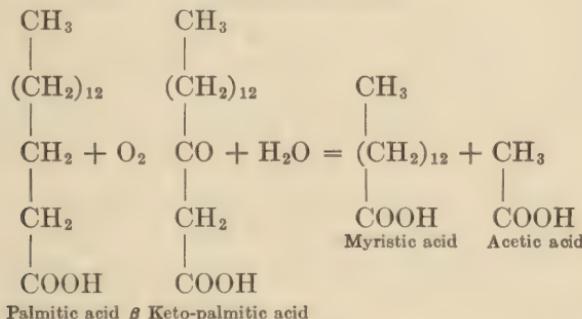


the most abundant of the normal fatty acids constituting nearly all the fatty acid groups of many plant and animal fats;



All of these fatty acids have a structure illustrated by the formulas for butyric and caproic acids, namely a straight chain of carbon atoms with one carboxyl group at the end. Although some of these chains are very long, yet every one of them is composed of an even number of carbon atoms. This is due to the fact that the process of fat synthesis in plants or animals involves a progressive building-up of the fatty acid chains by the addition of two links at a time. Each fatty acid molecule which serves as the starting point for the construction of a more complex one is carried through a synthetic process which adds two units to its carbon chain. Moreover, when fatty acids are destroyed by oxidation, in organisms, the point of attack is always at the β carbon atom resulting in the elimination of two carbon atoms from the chain and producing a new fatty

acid containing two less carbon atoms than the original. The process is illustrated, in the case of palmitic acid, thus:



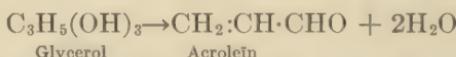
Many other fatty acids, in addition to the above list, are found in natural fats. Two are worthy of special mention: **Oleic acid**, $\text{C}_{17}\text{H}_{33}\cdot\text{COOH}$, the chief fatty acid of all the vegetable oils (olive, corn, peanut, etc.) and *linolic*, $\text{C}_{17}\text{H}_{31}\cdot\text{COOH}$, occurring in some vegetable oils (linseed). These and others of their type are known as *unsaturated fatty acids*. It is seen on comparison with stearic acid, $\text{C}_{17}\text{H}_{35}\cdot\text{COOH}$, that oleic and linolic acid have a comparatively deficient quota of hydrogen atoms in the molecule. This is explained by the presence of a double bond between certain carbon atoms, so that oleic acid is represented thus: $\text{CH}_3\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{COOH}$. The double bond is represented as occurring in the middle of the chain because certain types of oxidation cause oleic acid to yield two molecules of acids each with nine carbon atoms in its chain. Linolic acid is possessed of two unsaturated double bonds, while others with three or even four of them have been found in small amounts. The special physiological value of the unsaturated fatty acids is indicated by the fact that they are easily oxidized. They may even oxidize "spontaneously" as when exposed to sunlight or ultraviolet rays. Moreover, in the presence of reducing agents they readily take up hydrogen and are changed to stearic acid. This is the basis of the reaction in which vegetable oils, cottonseed, corn, etc., are hardened to produce the commercial lard substitutes. The same unsaturated carbon atoms, that can thus take up hydrogen, may also combine with other kinds of atoms or with groups; so that the resultant reactivity of unsaturated fatty acids renders them capable of functioning in organisms in ways that saturated fatty acids can not. The unsaturated carbon readily combines with iodine and the proportion of iodine, absorbed by the fatty acids of any fat, is a measure of the unsaturated fatty acids in it. This measure, the so-called iodine number, is of service in the identification of typical natural fats.

Palmitic, stearic and oleic acids are the most abundant fatty acids in nature. Indeed, they so markedly predominate in all naturally occurring

fats that the latter may be loosely defined as the palmitates, stearates and oleates of glycerol.

The properties of the two types of fatty acid, saturated and unsaturated, are quite different. The normal acids, with carbon chains twelve or more units in length, are solid at ordinary temperatures and boil only at high temperatures and diminished pressures. The lower members of the series are more like acetic acid, their prototype. They are liquid at ordinary temperatures and are comparatively volatile. The unsaturated fatty acids, however, though having long carbon chains, are oily liquids at ordinary temperatures, but are not readily volatilized. All the higher fatty acids, both saturated and unsaturated, are insoluble in watery solutions but are readily soluble in a long list of the so-called fat solvents: Ether, chloroform, carbon tetrachloride, benzine, gasoline, carbon disulphide, etc., also in alcohol which is not a very effective solvent for fats. The higher fatty acids burn like paraffin or fats. When in melted condition they spread over water like an oil. In short, their most prominent features, solubility, appearance and general physical properties, are those of fats.

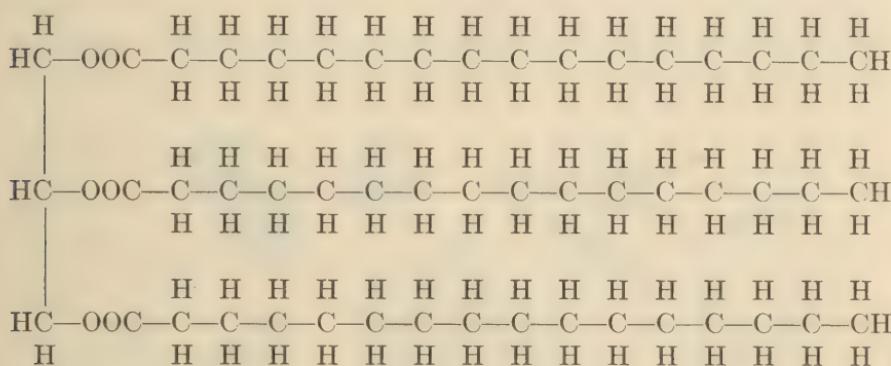
The Chemistry of Glycerol.—Glycerol, the other constituent of fat, is familiar as a clear, colorless, viscid, sweet-tasting liquid of the consistency of a thick syrup. It has none of the typical properties of fat. It is soluble in water and alcohol in all proportions but practically insoluble in ether and some other fat solvents. Its preparation from fat is possible after the fats are saponified, thus liberating glycerol and producing soaps from the fatty acids. It was the demand for glycerol to make nitroglycerine which caused the well known shortage of fats in some of the warring countries during the years 1916–18. Glycerine, either free or in combination with fatty acids in the fat molecule, gives a characteristic reaction when heated with potassium bisulphate, KHSO_4 . The latter serves as a dehydrating agent so that it removes two molecules of water from glycerol and liberates acrolein, a volatile substance with an unmistakable, irritating odor.



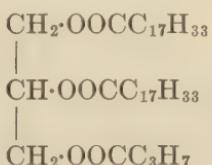
The acrolein test serves to identify glycerol and since the latter does not commonly occur in organisms except in the form of fats, the test may be used to detect them as well.

Structure of Fats.—Glycerol and fatty acids, when fed to animals or produced during intestinal digestion of food fats, are readily synthesized into fats in the absorbing cells which line the intestine. Presumably a similar synthesis occurs in plants. The result appears to be invariably a triglyceride, although a union of either one or of two molecules of fatty acid of glycerol may be made by artificial syntheses. No one fatty acid serves exclusively for the synthesis of fats in any plant or animal struc-

ture; so that all natural fats, as commonly obtained, are mixtures and not chemically pure compounds. By suitable processes of separation, however, it has been possible to obtain chemically pure fats and to show that, in some cases at least, fats constructed from glycerol and higher fatty acids have only one kind of acid in the molecule. They are accordingly named **triolein**, **tristearin**, **tripalmitin**, or **olein**, **stearin**, **palmitin**, etc. The formula is represented in the following manner, palmitin serving as an example:



The common abbreviated formulas are: Palmitin, $\text{C}_3\text{H}_5(\text{OOCC}_{15}\text{H}_{31})_3$, stearin, $\text{C}_3\text{H}_5(\text{OOCC}_{17}\text{H}_{35})_3$ and olein $\text{C}_3\text{H}_5(\text{OOCC}_{17}\text{H}_{33})_3$. In some fats, especially those containing groups from the lower fatty acids, more than one kind of acid appears to be present in the same molecule. These are termed *mixed glycerides*. For example, monobutyl-diolein of butter fat may be regarded as constituted thus:



Properties of the True Fats.—The solubilities of the neutral fats are their most striking characteristic. They are completely insoluble in water and, except for their different solubility in alcohol, have the same solubilities as the higher fatty acids.

The characteristic "fatty" or "waxy" texture would tend to make one suppose they could not be crystallized. The stearates and palmitates or fats predominating in stearin or palmitin, however, are quite readily crystallized. It is only necessary to dissolve them in ether, add an equal volume of alcohol and permit the ether and part of the alcohol to slowly evaporate. The fats are then thrown out of solution so gradually that they crystallize. The crystal forms consist of microscopic sheaves of needles closely resembling the crystals of palmitic or stearic acid obtained

by cooling their hot alcoholic solutions. Sketches of fat and fatty acid crystals are shown in Fig. 15.

The consistency of fats, whether they are semisolid like mutton tallow and bayberry wax, soft solids like butter and lard or oily like olive and cottonseed oil, is determined by the relative proportions of saturated and unsaturated fatty acid groups in their composition. Fats in which the palmitates and stearates predominate are comparatively hard at room temperature, while those composed chiefly of olein are oils. The softer solid fats contain more olein than the harder ones. The consistency is determined by the melting point so that the iodine combining power which, as mentioned above, is an index of the proportion of unsatur-

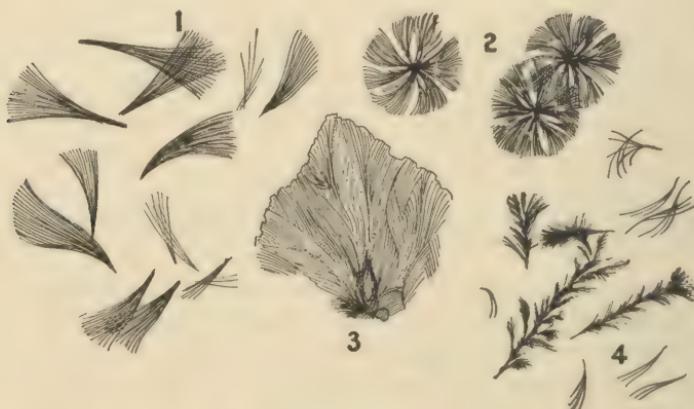


FIG. 15.—Sketches to show the microscopic appearance of crystals of fats and fatty acid: 1, from pork fat; 2, from beef fat; 3, from mutton fat; 4, palmitic acid.

ated fatty acid present, is comparatively high in fats which melt at low temperatures. The melting point of a fat varies, then, according to the relative proportions of its various fatty acids. Fats from different sources show a wide range of melting points and even the different specimens of fat from the same parts of animals of the same species do not always show uniformity. Lard, prepared from abdominal fat of pigs on diverse diets, may vary in melting point over a range of 5 or 10°C. This variability is indicative of the lack of constancy in the fatty acid components of natural fats. Since melting points of fatty acids determine those of fats, figures for the more important fatty acids are given.

FATTY ACID	MELTING POINT
Lauric.....	43.6°C.
Myristic.....	53.8°C.
Palmitic.....	62.6°C.
Stearic.....	69.3°C.
Oleic.....	14.0°C.

Linseed oil which, because of its high content of unsaturated fatty acids, hardens on exposure to air and sunlight and is therefore an important constituent of paint, remains an oil on cooling even to temperatures as low as -28°C .

The yields of the various fatty acids, obtained from some typical specimens of butter fats, are shown in the table below.

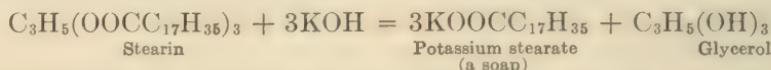
Acid	Fatty acids, per cent	Equivalent triglycerides, per cent
Dioxystearic.....	1.00	1.04
Oleic.....	32.50	33.95
Stearic.....	1.83	1.91
Palmitic.....	38.61	40.51
Myristic.....	9.89	10.44
Laurie.....	2.57	2.73
Capric.....	0.32	0.34
Caprylic.....	0.49	0.53
Caproic.....	2.09	2.32
Butyric.....	5.45	6.23
Total.....	94.75	100.00

Because of the difficulties involved in quantitative separation, analyses of the fatty acid content, to show the proportions of the individual acids, have not been extensively made.

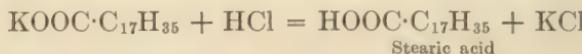
The fats, like the fatty acids, have a specific gravity lower than that of water so that they float on watery solutions. The extremely thin layers into which oils spread on water surfaces are phenomenal. By interference of light waves iridescent colors are produced on such films and permit physical measurement of their thickness. The resulting figures indicate that the film may in some cases consist of a single layer of molecules.

The saponification of fats is really a form of hydrolysis and indeed fats may be split by all the usual hydrolytic agents: Water, steam, boiling acids and alkalies, and suitable hydrolytic enzymes. The result is, in general, glycerol and fatty acid; but if cleavage occurs in an alkaline medium, the base, which furnishes the alkalinity, combines with fatty acid to form soap. Soaps may be defined as the metallic salts of the fatty acids. The splitting of fats is greatly facilitated by soap formation; so that without the presence of an alkali, the amount of hydrolysis of any fat is almost negligible, even though a fat hydrolyzing enzyme be

present. The reactions occurring in saponification may be represented by the typical one between stearin and potassium hydroxide.



All soaps react with mineral acids to liberate fatty acids. Thus:



Since soaps formed with alkali metals are soluble in water while fatty acids are markedly insoluble, a substance may be tested for fat in the following manner. Boil with dilute KOH, evaporate to dryness, extract with water and acidify the filtered extract with HCl. A white precipitate of fatty acid is an indication of fat.

Properties of Soaps.—Soaps have a remarkable power of facilitating the emulsification of fats. Any oily fats thoroughly shaken with water tend to subdivide minutely into microscopic droplets which, suspended in the water, constitute an emulsion. This condition, however, is very transient if only fat and water are used. Separation into water and an oily layer quickly follows. But if a small quantity of soap is dissolved in the water, emulsification may result from a mere gentle agitation and be maintained with comparative permanency. Since protoplasm behaves in certain respects like emulsions, the relation of fats and soaps to their production is of fundamental physiological significance; but as lipoids, proteins and inorganic constituents are also concerned in the production of emulsions, it is advisable to reserve discussion of the process until later.

The usefulness of soap in cleaning is due to its fat emulsifying power, since in that way it loosens dirt. A soap, to be an effective cleanser, must be in solution and the reason why so-called hard waters are ineffective in washing is that they contain salts which precipitate soaps. All the alkali earth metals and most of the heavy metals form insoluble combinations with fatty acids. The only soluble soaps are those of the alkali metals. Commercially, those of sodium are called hard soaps, those of potassium, soft soaps, though the hardness of a given commercial preparation is also dependent on the amount of water in it. Soap mixed with hard water produces a milky appearance, which may change to a flocculent white precipitate. Calcium and magnesium salts in such water react with soluble soaps to form the comparatively insoluble calcium and magnesium soaps composing the precipitate. A practical measure of the amount of hardness of water, designed for domestic or industrial use, is obtained by measuring the amount of a standard soap solution required to enable a measured quantity of the water to give a permanent soapy foam when shaken. Foaming indicates soap in solution and so shows the end point of the precipitation of calcium and magnesium soaps.

Distribution of Fats.—The neutral fats are widespread in nature. It is possible that they are represented in the composition of every plant and animal tissue. The amount in the protoplasm of very active cells, however, must be very small—not more than a mere trace, if present at all. Very active cells include those of skeletal and heart muscle, of leaves and the growing buds of plants. Fats are not abundant in the hardened tissues of animals and the woody parts of plants. Accumulations of neutral fat are found, however, in what might be called the storage parts of plants and animals: In seeds, fruits and nuts, and in adipose tissue of animals. Adipose tissue is found in the subcutaneous and intermuscular regions and is especially apt to accumulate in the abdominal region forming, among other deposits, the *panniculus adiposis*. Animal fat also occurs in considerable amounts in liver and bone marrow, and to a lesser degree in nervous tissues which, as will be explained below, are composed chiefly of lipoids. True fats seem to be oxidized and so to disappear from active cells about as rapidly as they are taken in. Accumulation in non-active tissues is an index of the most important usefulness of fats, namely, to serve as storage food.

The ordinary method of estimation of the fat content of tissues or foods is complete extraction of the dried material with ether or other fat solvent and weighing the extracted fat after evaporation of the solvent. Experience with this method has thrown some light on the nature and behavior of tissue fats. It has been found that preliminary drying must be carried on at low temperatures or during exclusion of air to prevent partial oxidation and consequent increase in the weight of the fats. This is an indication of their content of unsaturated fatty acids. Tissues, repeatedly and exhaustively extracted with ether, if then subjected to a prolonged action of protein hydrolyzing enzymes and again dried and extracted, yield further amounts of fat. This is taken to indicate that some tissue fat is held in an unknown chemical combination with protein and is liberated only when the latter is destroyed.

The fatty substance, obtained in an extraction of tissue or food material, is called crude ether extract and in reality contains various things in addition to true fat. They are named collectively the lipoids and will be described presently. It is possible to make a laborious separation of the true fats from lipoids, but analytical figures as ordinarily given are percentages of crude ether extract obtained from the dried material. For this reason an appreciable quantity of fat is reported in the analysis of all tissue materials and of the majority of foodstuffs.

The Origin of Fats in Plants.—The processes of fat production in plants, unlike those of carbohydrate production, do not appear to be directly dependant on photosynthesis. Fat is found in colorless plants, such as bacteria; but proof that it is actually produced in them, in contrast to mere storage, is not complete. All great accumulations of fat as seen

in the plant world are found in green or, at least, in pigmented plants; so that fat production may be indirectly dependant upon photosynthesis. An interesting case is that of certain *diatoms*, microscopic algae, which contain chlorophyll or similar pigment. In them starch or other carbohydrate synthesis is at a minimum while the droplets of fat as seen in microscopic examination, though actually small, are really large in comparison with the total bulk of these minute organisms. Diatoms with fat droplets are shown in Fig. 16. Myriads of diatoms are found in the ocean and since they serve as the chief food of many small animals which in turn are eaten by larger marine fauna and since diatoms utilize very simple materials as food, the synthetic powers of these humble plants are

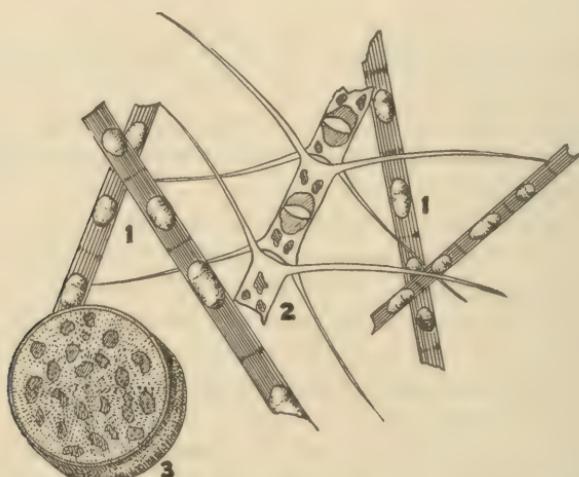


FIG. 16.—Some examples of marine algae which effect fat synthesis: 1, *Skeletonema*; 2, *Chaetoceros*; 3, *Coccinodiscus*.

of prime importance in what has been termed the "metabolism of the sea." Oils prepared by extraction of masses of diatoms have the characteristic odor of fish oil so that even the large carnivorous fishes doubtless make use of the same fats synthesized in the minute algae. Fat synthesis in marine plants plays a rôle in aquatic life somewhat analogous to that of photosynthesis of carbohydrates by green plants in terrestrial life. Production of fats in greater amounts in pigmented plants than in others strongly suggests that the fat is formed from carbohydrates.

Origin of Fats in Animals.—Extended studies have been made to determine what foods produce fat in animals. Actual storage of food fat is quite obvious even to casual observation. More decisive evidence is obtained by feeding animals on a diet rich in some fat with characteristics (melting point, content of peculiar fatty acid, etc.), distinctly different from those of the animal's body. Linseed oil, fed to dogs, can be actually

traced to the depots of fat storage in the animal. Curiously enough, however, fats are not stored in the body without some modification. Dogs, fed on a diet containing no hard fat but only oils, still maintain a texture in their storage fats harder than that of oils, but do show some tendency to acquire a store of fats of lower melting point than that of normal dog's fat. Similarly, the feeding of hard mutton fat has only a slight tendency to modify the texture of the fat of an experimental animal with naturally soft fat. In short, animals store their food fats, but can modify them somewhat during the process and always tend to preserve, within certain limits, the uniformity of the composition of their fat store.

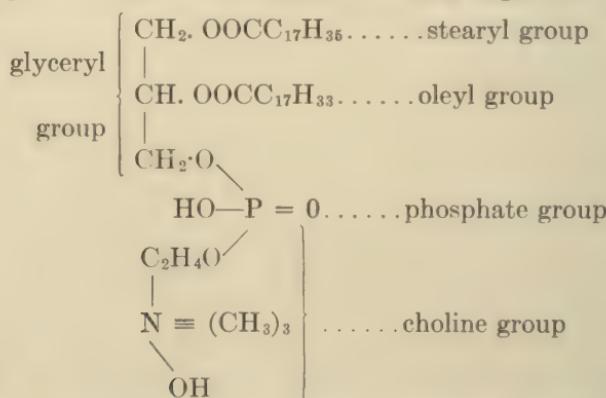
Transformation of carbohydrates into fats undoubtedly occurs in animals. The fattening effect of foods rich in starch and sugar is familiar in human nutrition. Agricultural practice, in fattening hogs and other domestic animals, recognizes carbohydrate foods as fat producers. Quantitative proof has been abundantly supplied. One type of experiment is based on actual analysis of body tissues. Of a large litter of pigs, for example, one half their number, killed at an early age, may be completely extracted to determine the total crude ether extract. The other half of the litter, maintained on a diet of known composition during a suitable period, may then be similarly treated. Increase of fats during the feeding period is compared with the total intake of food stuffs and is found to be so large that only the abundant carbohydrate material, used in such diets, could possibly serve to produce it. Experiments on cows have also shown fat synthesis from carbohydrate, since the milk fats, produced during a given period of observation, are far too great to be formed out of the non-carbohydrate material in the diet of a composition known and controlled during the entire period.

Production of fat from proteins in animals is more difficult to prove. Elaborate observations, designed to show that the total milk fat production of cows was greater than could be produced from the fats and carbohydrates of their food, have yielded results of such a questionable character that they have been interpreted both as proof and as disproof of the synthesis of fats from proteins. The computations are very complex and may be made in several ways; but in any case, they give no indication of a pronounced tendency of the animal body to change protein to fat. Certain proteins, it will be recalled, yield sugar on hydrolysis. Other proteins may serve as a source of sugar since it has been shown, especially by Lusk, that certain of the amino-acids may, under some circumstances, be transformed into glucose. Through the carbohydrate stage, then, proteins may, to a very limited extent, serve as fat producers in animals. Ability to produce fat from proteins has been proved to be present in developing fish eggs and in fly larvae. In the majority of animals, however, proteins are a minor source of fat.

THE LIPOIDS

The lipoids are an ill-defined group. The term is variously used. Signifying fat-like it would seem to apply to all substances, other than true fats, with properties similar to those of fats. By common consent it covers (1) the phospholipins and (2) the cerebrosides. The sterols, cholesterol and related compounds, have been classed with lipoids because of similar texture and solubility; but chemically classified, they would fall in a group by themselves.

The phospholipins (or phosphatides) are the phosphorus-containing fats. They are sometimes called the *lecithins*, since lecithin is the most abundant and well known member of the group. It is prepared by treating an ether-alcohol extract of any plant or animal tissue (brain or egg yolk give the largest yields), with an excess of acetone. Lecithin and some other phospholipins are precipitated since these substances, unlike most fatty materials, are comparatively insoluble in acetone or even in mixtures of it with other fat solvents. Crude lecithin, thus obtained may be purified by virtue of the insolubility of its cadmium salt. Lecithin has a peculiar behavior toward water. It very readily emulsifies to form, especially in the presence of an emulsifier, a well dispersed colloidal solution whose particles are irregularly shaped instead of being spherical and are called myelin forms. Lecithin, in short, forms a coarse colloidal solution in water. Subjected to hydrolysis, as by boiling with barium hydroxide, it yields fatty acids, glycerol phosphoric acid and a nitrogenous base, choline. Choline is a complex amine. Similar treatment of cephalin (also written kephalin) which generally occurs along with lecithin, yields the same products except that oxyethylamine is found instead of choline. From the way in which lecithin hydrolyzes, its molecular structure has been formulated. The glyceryl group is represented as attached to two fatty acid radicals. The third combining power of the glyceryl group is engaged with a phosphate group, which, in turn, is connected with the choline group. A theoretical formula for lecithin is given as follows:



Lecithin, mono-stearyl-mono-oleyl-glyceryl-choline phosphate.

This structural formula is in agreement with all the facts known about lecithin and the only step remaining to complete its proof is artificial synthesis. Although substances related to lecithin have been so produced, synthesis of true lecithin has not been attained. Stearic acid and oleic acid have been positively identified among the hydrolytic products of pure lecithin. Other fatty acids in place of these are said to occur in some lecithins. In every case, however, one of the fatty acid constituents is unsaturated. This component probably confers upon lecithin its marked tendency to spontaneously oxidize once it is separated from the tissue in which it occurs. It darkens to a brownish color on exposure to air and meanwhile shows a progressive decrease in its iodine absorbing power (p. 59). The readiness with which lecithin oxidizes is generally regarded as of significance for utilization of fat in living cells. There is evidence indicating that fat may be transformed into lecithin before oxidation. The choline group has aroused considerable interest since choline, trimethyl-oxyethyl-ammonium hydroxide, is a very poisonous substance. It has been detected in a free state in the cerebrospinal fluid of patients suffering from certain degenerative nervous diseases.

The phospholipins are classified according to the number of P and N atoms in the molecule. The usual grouping is as follows:

Phospholipins:

Monoamino monophosphatides, contain one nitrogen atom and one phosphate group; *lecithin* and *cephalin*.

Diamino monophosphatide; *sphingomyelin* of brain tissue.

Numerous other phosphatides have been described and alleged to occur in different animal and vegetable tissues. More extended observations have shown in several cases, however, that supposed new varieties were really mixtures of previously known ones or of phospholipins with other substances. A general simplification of the group has resulted. The seemingly established chemical individuals are the three mentioned in the above list.

The phospholipin obtained from the anterior lobe of the pituitary gland and named *tethelin* by Robertson, its discoverer, is of interest because of its curious effect on growth. Its physiology will be discussed in Chap. XXV. Its chemistry is still somewhat fragmentary.

A substance called *jecorin* obtained from liver and some other animal tissues appears to be a mixture containing phospholipin. It has much the same behavior as lecithin, but yields the sugar, glucose, on hydrolysis. It is found, however, that if to an ether solution of lecithin, one adds glucose which is ordinarily insoluble in ether, it slowly dissolves forming a sort of loose compound with lecithin. The combination is precipitated by acetone but it is not very stable since reprecipitations do not give constancy of composition of the successive precipitates. This artificial

combination of sugar and lecithin, then, may be regarded as a physico-chemical compound. It has much the same behavior as jecorin from the liver. *Cuorin* of heart muscle seems to be a mixture of the decomposition products of cephalin and lecithin. A preparation called *protagon*, made from nervous tissue is probably a mere physico-chemical union of the phospholipin, sphingomyelin, with cerebrosides (see p. 71). *Protagon* crystallizes so readily and perfectly upon cooling of its solution in 85 per cent alcohol and from some other solvents that it has been considered by several by its investigators to be a distinct chemical compound. But recrystallization does not always produce a substance of the same composition; it does so, in fact, only when the conditions under which it first crystallized are very faithfully reproduced. For this reason it is regarded by some investigators as a mere association of lipoids rather than as a unit substance.

The problem, that of distinguishing between mixtures and compounds, is frequently encountered in physiology. With complex colloids, proteins, fats, lipoids, carbohydrates, such a variety of combinations may occur—some so loosely made that one substance may be said to merely dissolve another, as in the case of lecithin acting on glucose to form jecorin—that the physiologist can not always draw sharp distinctions between associations of substances and their loose chemical combinations. Indeed protoplasm itself, all the protoplasm in a cell, has such a close association of its many colloidal and other components that it behaves in certain ways as a unit substance. For example, a reaction, aroused by a stimulus acting on any part of a living cell, may spread so as to involve the entire cell. This and other observations led to a “theory of the biogen molecule,” which supposed that all the material composing a living cell was loosely cemented together, chiefly by the diverse combining powers of proteins, into one huge, unstable molecule. This theory obviously pretended to nothing but a very crude conception. Current theories on this subject are still unsatisfactory, but lay more emphasis on the looseness of such chemical associations by calling them physico-chemical combinations. The history of jecorin and *protagon*, described first as chemical compounds, later as mere physical mixtures and still later as physico-chemical combinations, is typical, perhaps, of the development of many similar problems.

It will occur to the reader that the distinction between a true chemical compound and a physico-chemical one is rather arbitrary in the case of complex colloids. This is particularly true of the amphoteric colloids since they have complex combining powers. Lecithin is amphoteric, owing to the replaceable hydrogen in its phosphoric acid group and the replaceable —OH in its basic choline group. (See formula.) The other phospholipins are also amphoteric; so that the whole group, like the amino-acids and the proteins, shows diverse combining powers.

Phospholipins occur, probably, in every living cell. Although several different ones may be found in the same cells, lecithin, or possibly a cephalin, at least a lecithin-like substance, has been found in every plant and animal tissue that has been adequately examined for phospholipins. Nervous tissues contain the greatest abundance; but liver, muscle tissue, bile and egg yolk are also abundant sources. It has been claimed, with good analytical proof, that every cell and tissue contains a constant, characteristic, and, with the possible exception of liver tissue, unvarying proportion of phospholipins.

The Cerebrosides.—The cerebrosides, so named because they are abundant in nervous tissue, are a distinct group of lipoids. They are sometimes called the *glycolipins* since they are compounds of a sugar group with a fat-like complex. They are readily prepared in crystalline form by cooling a hot alcoholic extract of desiccated brain tissue. Complete separation and purification is, as in the case of all the lipoids, a process of some technical difficulty. Largely because of this, the structure of the cerebrosides is not entirely established. They contain one atom of N but no P. Subjected to hydrolysis they yield the sugar, *galactose*, together with *fatty acids* and *sphingosine*. The latter is a complex alcohol containing an amino group. The two cerebrosides which have been distinguished, *phrenosin* and *cerasin*, differ as to the fatty acid in their complex; the first yields *cerebronic acid*, the other *lignoceric acid*.

The Sterols.—The word sterol means solid alcohol. The sterols are widely distributed in nature. Because their solubilities are similar to those of fats they have been classified with the lipoids. But they are really alcohols and not chemically related to fats. *Cholesterol*, formerly called cholesterin, is the best known member of the group of sterols. Its formula, $C_{27}H_{46}OH$, shows one—OH group indicative of an alcohol. Although its structural formula is not yet completely established much of the complex atomic grouping in it has been deciphered. It belongs in the group of *terpenes* which also includes some of the other sterols. Terpenes also include cholic acid. Its significance as a component of bile will be discussed later (Chap. XVII). Cholesterol does not readily decompose. It resists all biological agents, except a few species of bacteria, and all chemical agents save concentrated mineral acid or powerful oxidizing reagents. It is a very stable substance.

Cholesterol appears to occur in every animal cell, also in blood, lymph and bile. It occurs abundantly in gall stones which are usually the result of crystallization of cholesterol from the bile.

It is easily prepared by crystallization from its solution in alcohol, ether or other fat solvent. The characteristic rhomboidal plates may be either microscopic or macroscopic. They are waxy and nearly transparent (Fig. 17).

Cholesterol gives a number of characteristic color reactions when subjected to certain procedures: (1) A cherry red with a greenish fluorescence when its chloroform solution is treated with concentrated sulphuric acid (Salkowski test); (2) a violet color, changing to bluish green when its chloroform solution is treated with a few drops of acetic anhydride and then with concentrated sulphuric acid (Liebermann-Burchard test); (3) a violet color of the dried residue when solid cholesterol is moistened with concentrated hydrochloric acid containing ferric chloride and the resulting mixture evaporated to dryness (Schiff test). From its alcoholic solution cholesterol is precipitated by the alkaloid digitonin.

The latter reaction is the basis of a method for quantitative estimation of cholesterol. Although such estimations reveal the presence of cholesterol in all animal tissues and fluids, they

show it to be more abundant in nervous tissues than elsewhere. Many determinations on the human brain show an average content of about 2.7 per cent of cholesterol in the fresh, undried tissue of the whole brain. Its amount is variable in different specimens of bile, normally about 0.1 per cent; but in bile from a gall bladder, as much as 4.7 per cent has been found. In blood and in tissues other than nervous, a small amount, less than 0.1 per cent is found.

Some derivatives of cholesterol occur in the excreta and in the tissues of animals. Koprosterol or stercorol, $C_{27}H_{48}O$,

in human feces and hippo-koprosterol, $C_{57}H_{54}O$, in horse feces seem to be the result of bacterial reduction of cholesterol and other sterols in the intestine. Cholesterol, being an alcohol, can unite with organic acids and is frequently found in union with fatty acids. Such compounds are called cholesterol esters. They are widely distributed in animal tissues and fluids. A large part of the cholesterol of blood is in this form. The secretions of the skin contain cholesterol esters. Lanolin prepared from wool fat is a cholesterol ester, useful as a salve.

A group of compounds often confused with cholesterol and nearly identical with it have a widespread distribution in plant structures. Some representative of the group occurs, apparently, in every plant cell. These compounds are called *phytosterols* (solid alcohols of plants). A number of plant sterols have been described, some isomeric with cholesterol, others less closely related. Details of their structure and biochemical behavior have not been investigated as completely as have those of

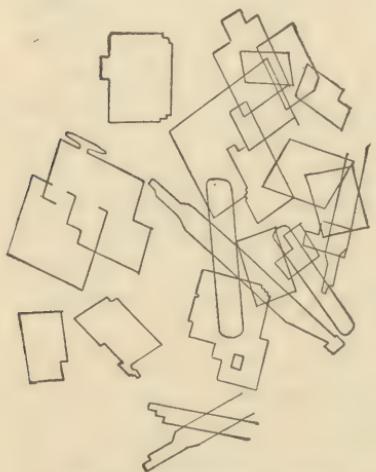


FIG. 17.—Cholesterol crystals.

cholesterol; but it seems clear that, in plant cells, they take the place filled by cholesterol in animal structures.

THE RÔLE OF FATS AND LIPOIDS IN LIFE

Energy Value of Fats.—The chief function of the true fats is to furnish a concentrated store of fuel of high energy value. Packed away in the storage cells of plants and animals, both the solid fats and the oils contain more potential energy than could be held in the same bulk in the form of other biochemical substances. Used as food fats, they are pre-eminent sources of energy. All energy values, it should be recalled, may be translated into heat producing power, and since heat is measured in

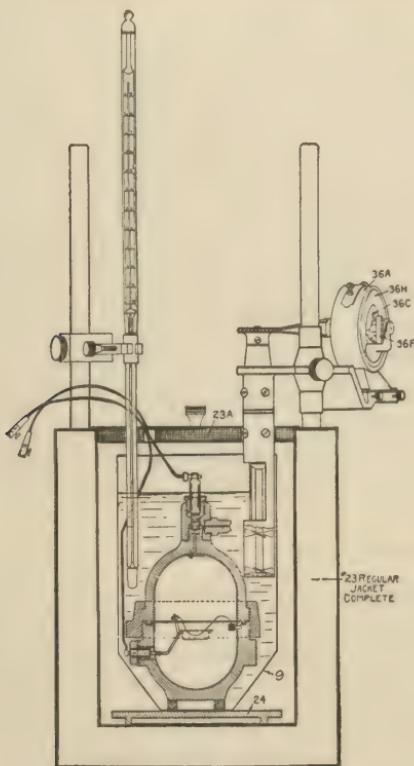


FIG. 18.—Bomb calorimeter. The steel bomb is shown in sectional view. The crucible, with electrical connections for making an arc, is supported in the center of the bomb. The water which surrounds the bomb is kept in motion by a stirring device actuated by a small motor. (36 A-F) The bulb of a delicate thermometer is immersed in the water. The jacket and cover which form the outside container are constructed so as to afford heat insulation. (As supplied by the Emerson Apparatus Co.).

units called calories, the measurement of energy is called *calorimetry*. A calorie is the heat required to raise 1 gram of water through 1°C . A calorimeter is illustrated in Fig. 18. The actual heat evolved in burning one gram of fats is 9.3 calories which is more than twice

as much as the corresponding value for carbohydrates (4.1 calories). Since both fats and carbohydrates are oxidized to CO_2 and H_2O in the body as well as in a flame, the calorimetry values for fats and carbohydrates are also their physiological fuel values. Caloric values of fat are nearly twice those obtained as an average for protein. Taking into account the fact that proteins are not completely oxidized in the body but are changed into urea and other nitrogen-containing wastes which still have considerable potential energy, that is, can be further oxidized, it has been shown that, for physiological purposes, fats have more than twice the heat value of proteins. It is true that fats may be poorly digested or may escape oxidation in an animal body suffering from one of several disorders of metabolism. Fat, indeed, is not so readily utilized as are carbohydrates and proteins. But in the normal healthy animal, fat is burned; so that its high heating value is of real significance. Reports from all who have observed the eating habits of the Eskimos tell of their great preference for fat. They will consume a huge mass of whale blubber, solid fat, with all the relish shown by a child of temperate regions while eating candy. The need for food of high fuel value finds expression in the appetite of these natives of very cold countries.

Neutral fats accumulate only in the plant and animal structures which may be termed storage regions. The reserves of fat are utilized during a period of food scarcity. During starvation or during inadequate feeding the adipose tissues of animals are the first ones to show marked losses. The store of fat in seeds, nuts, etc. is useful to meet the high energy requirements incident to rapid growth and development during germination.

Fats also possess a high *heat insulating power*. They are not good conductors of heat. A layer of subcutaneous fat, then, furnishes an efficient heat-conserving blanket for the warm blooded animals. This is a marked characteristic of whales and all other cetacea which must maintain a comparatively high temperature even in cold waters. Fish, on the other hand, do not maintain a temperature above that of their surroundings and possess comparatively little subcutaneous fat.

Lipoids in Cell Membranes.—The functions of lipoids are much more numerous and complex than those of true fats. Some representative of both lecithins and sterols may be found in every cell. This seems of itself a significant fact. A study of the composition of erythrocytes (the red corpuscles of blood) has added another significant point. Lecithin and cholesterol are found in the stroma which comprises the outside envelope of these cells. It is possible to separate stroma from the rest of the corpuscle. For this purpose, a mass of corpuscles, separated from blood by centrifugation, is stirred with water. Under this treatment, the corpuscles burst (hemolysis) and their contents freely dissolve in the water (Fig. 19). The capsules or stroma, however, remain undissolved appearing under the microscope as the socalled "ghosts of corpuscles"

and may be collected by centrifugation of the watery solution of the corpuscles. Although stroma constitutes only a very small part of blood cells, enough may be collected in this way to permit of analysis. It is found that lecithin and cholesterol compose nearly 40 per cent of stroma. Protein and inorganic material constitute the remainder. From this and other observations it has become apparent that lipoids are extremely valuable as components of membranes of living cells.

The belief that matter can only be alive when enclosed in protective cell membranes has become more and more firmly established by all the developments of physiology since the statement of the cell theory (1833-1839). Fundamental qualities of living matter are dependent

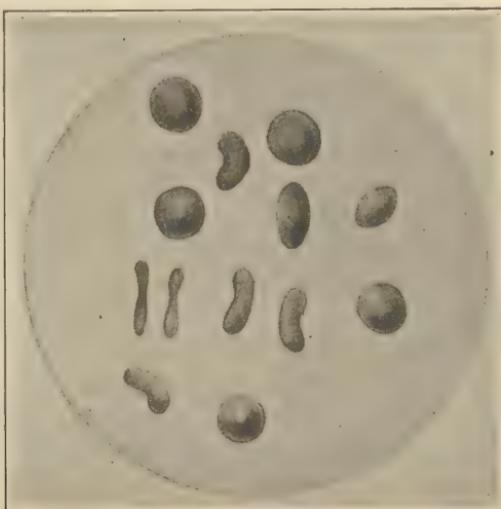


FIG. 19.—Blood corpuscles, some of which are swelled in initial stages of hemolysis.

upon some protection from the environment. Metabolism is constantly proceeding, so that the chemical composition of living matter is ever changing. Yet these changes must be largely reversible; for the chemical composition of every bit of protoplasm varies only within the narrow limits which permit the maintenance of that integrity of composition, consonant with the highly developed individuality of every different kind of living structure. Maintenance of such integrity demands that protoplasm shall constantly "select" its specialized requirements and "reject" other materials, while providing, simultaneously, for ejecting its equally specialized products and wastes. Free diffusion between protoplasm and its environment spells instant death.

The protoplasmic covering that thus appears to "discriminate" delicately is no mere dead partition but must be constituted with a cunning intricacy beyond the conception of present day science. The limiting membrane of protoplasm must be the very guardian of life.

Hoping eventually to understand the nature of living matter, one finds no problem more alluring than those relating to the nature and behavior of cell membranes. No wonder, then, that a considerable portion of physiological research in recent years has been devoted to such studies.

The presence of lecithin and cholesterol in so-called cell membranes seemed a promising clew to the mystery of their behavior. Lecithin, being a fat, does not form a true solution in water; but being a peculiar phosphorized fat, it does have a kind of affinity for water. It is readily dispersed, in colloidal form, in water or watery solutions. Cholesterol, on the other hand, is a highly stable, semi-solid, waxy material, quite insoluble in water and as impermeable to it as is shellac. The idea is suggested at once that a suitable combination of lecithin and cholesterol might give a satisfactory degree of impermeability against certain constituents of the watery environment, which in the form of blood, lymph, plant juice, soil water, sea water or some other medium surrounds all living cells in any state approaching maximum activity.

It should be clearly understood that the heavy supporting walls of cellulose in plant structures and many other clearly visible partitions in tissues are not referred to when we speak of the true limiting membrane of protoplasm—the membrane which has to do with the selective power of living matter. The comparatively heavy walls are really deposits of dead matter and are as freely permeable to all molecules of sufficiently small size as parchment or collodion would be. The true living membranes are too thin and transparent to be visible under the microscope. Even the stroma of corpuscles can not be seen until the cell is ruptured.

It is, nevertheless, highly probable that every protoplasmic mass does have a distinct limiting membrane. Not only the general discriminating behavior of living matter, mentioned above, but also numerous physico-chemical facts testify to its presence. The phenomena of surface tension furnish one of the best proofs. This force, to be described presently, exists at the surface of any solution and so operates that it draws many substances in solution, and especially lipoids, to the surface so as to make them more concentrated there than elsewhere in the solution. Every kind of protoplasm is a solution and contains lipoids, so that we are justified in assuming a tendency to form a concentration of lipoids, along with other constituents, at protoplasmic surfaces which we call limiting membranes—even though they may be of an ultramicroscopic thinness.

The Lipoid Theory of Cell Permeability.—Many efforts have been made to establish the truth of what has been termed the lipoid theory of cell permeability. Overton has been a leader in such work, so that the theory is often named as his. Briefly stated, it is: Any substance having solubilities similar to lipoids and tending, therefore, to dissolve in a colloidal solution of lipoids may diffuse into or out of living cells; while other substances can not cross their limiting membranes. Although this theory

expresses only a partial truth, yet so many observations tend to support it that the evidence is worthy of examination. Experiments with a variety of plant and animal cells show, that, while impermeable to very dilute NaOH, they are permeable to corresponding strengths of NH₄OH. One demonstration of this fact is seen in the following experiment: Leaves of an aquatic plant are placed in a very dilute solution of the dye, neutral red, until a concentration sufficient to color the cell contents has been taken up by the protoplasm. Neutral red is red in faintly acid or neutral solutions but yellow in alkaline solution. If some of the stained leaves are put into very dilute NaOH the red color persists but those in dilute NH₄OH turn yellow. Ammonia is soluble in lipoid solvents, benzene, chloroform, ether etc., while NaOH is not.

Moreover, extended experiments with aniline dyes have shown that those which, like neutral red, are soluble in fat solvents penetrate the cells while other dyes, insoluble in such reagents, do not enter living cells. Experience with the dye, methylene blue, is instructive. It is insoluble in chloroform but does dissolve in chloroform containing a lipoid. Methylene blue penetrates living cells.

It has also been shown that fatty acids, acetic and butyric, which dissolve in lipoid solvents or in lipoids themselves, are more effective in influencing certain cell processes than are corresponding concentrations of acids not showing such solubilities as, for example, hydrochloric or other mineral acids.

Although such observations indicate that lipoid solubility plays a part in some phenomena of cell permeability, yet inorganic salts, sugar, amino-acids, etc. which are not "lipoid soluble" and to which cells show impermeability under some circumstances, do penetrate cells in some unexplained manner. Certainly salts, sugar and amino-acids are indispensable as foods to maintain the very life of cells. Moreover sugar and amino-acids injected into the circulating blood quickly disappear and, shortly after injection, are found to be present in various animal cells, such as liver, muscle, and blood corpuscles. Clearly, a mere lipoid membrane at the surface of cells is utterly inadequate to account for the peculiar selective powers, this seeming discrimination, of protoplasm.

Surface Tension as a Membrane-forming Force.—An understanding of the forces responsible for the production and maintenance of protoplasmic membranes would help in explaining their composition and physiological behavior. One of these forces, surface tension, has been extensively studied. It is defined as the strain or tension upon the surface of a substance in contact with another substance with which it does not mix. If both immiscible substances are liquids each has its own surface tension at the interface. Gases, because of the freedom of movement of their molecules, do not have surface tension and solids do not lend themselves to a measurement of their surface tensions.

One of the many possible ways by which the existence of surface tension is made apparent is the flotation upon the surface of water of small substances of high specific gravity. Dry sand or dirt, if gently sprinkled upon the surface, readily floats until by some chance the surfaces of the particles are wetted, when they sink as rapidly as they would if at first introduced below the surface of the water. Dry particles of methylene blue are useful in the demonstration; because one can follow the progress of the solution of the dye in the water and can observe that when enough of it has been dissolved to lower the surface tension in the immediate neighborhood of the dry grain of powder, it suddenly drops through the water surface as though a trap door had been opened and then sinks

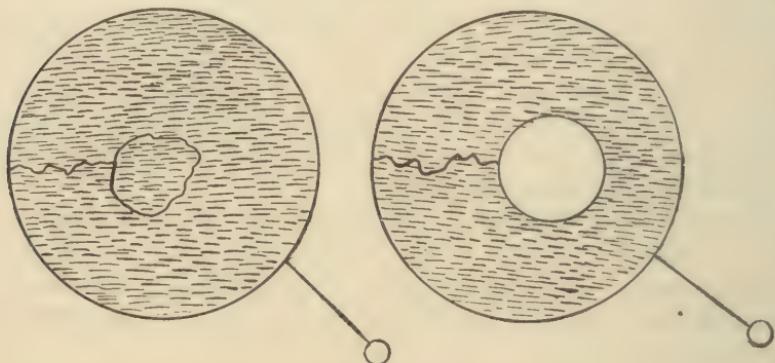


FIG. 20.—Experiment to show the pull of surface tension. A loop of silk thread lies upon a soap film which stretches across a circle of wire. After the film within the loop of thread has been broken by touching it with the tip of a piece of filter paper, the loop is drawn into a perfect circle by the tension of the remaining film. (After Van der Mensbrugghe).

slowly to the bottom. Small insects, of specific gravity greater than water, are floated by surface tension.

The origin of surface tension is molecular attraction or what is known as "internal pressure." All the molecules of any liquid attract each other with a cohesive force sufficient to maintain the liquid condition. If the cohesion were less, the substance would become a gas; if more, the comparative freedom of molecular motion would be lost and solid consistency would supervene. Each molecule not at the surface is about equally attracted by the surrounding molecules in every direction; but those at the surface are subject to an unbalanced condition of forces. They are therefore under a peculiar condition of strain or stress resulting in behavior of the surface similar to that of a stretched elastic membrane.

The Principle of Minimal Surfaces.—In consequence of this the surface of the liquid always tends to become the least possible, in other words to draw itself in. This is apparent upon observation of the remarkable behavior of a liquid suspended in another immiscible liquid of the same specific gravity. Under these conditions all the pull of gravitation

upon the suspended liquid is equalized and prevented from affecting its form. It will assume the shape of a perfect sphere, since that is the body possessing the least surface for a given volume. The experiment is easily made. Drops of oil are put into 60 per cent alcohol and either 70 per cent or 50 per cent alcohol is added until the liquid has a specific gravity which permits oil drops neither to rise nor fall. If oil is then allowed to flow from the tip of a pipette, introduced below the surface of the alcohol, a sphere several inches in diameter may be formed. If it is pushed or pulled out of shape or in any way deformed it immediately resumes its perfect spherical shape. The surface pull is also shown in the experiment with a soap film illustrated in Fig. 20.

Measurement of Surface Tension.—Surface tension may be measured by the use of the phenomenon of *capillarity* which is caused by it. If

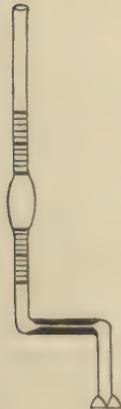


FIG. 21.

FIG. 21.—Stalagmometer. The graduations permit measurement of the amount of liquid flowing out. The section of fine capillary tubing insures that the outflow shall be in drops. The flat surface surrounding the small opening at the bottom serves for the formation of drops.

FIG. 22.—The Searle torsion balance. Explanation in text. (*As supplied by Pye and Co., Cambridge, Eng.*.)

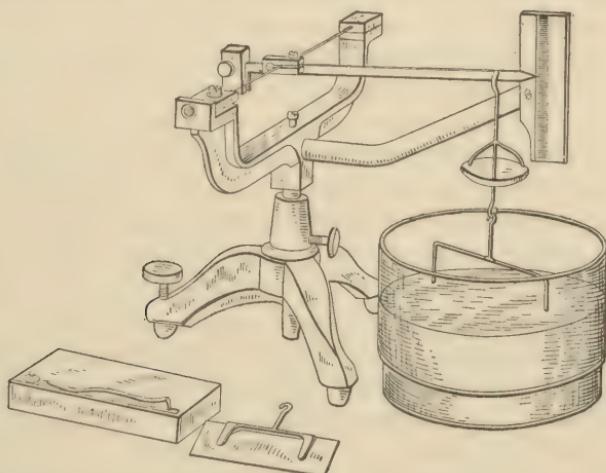


FIG. 22.

the tension of a liquid at its air surface is greater than at surfaces in contact with a solid, the liquid tends to flow over the solid. Glass is a suitable solid for use in such observations. Water and most watery solutions flow over glass or, as frequently expressed, "wet glass." For this reason they rise in capillary tubes of glass to a level, whose height, above the main level of the liquid outside the tube, is determined by the diameter of the tube, the specific gravity of the liquid, and its surface tension. In a given tube under standard conditions, liquids of approximately the same specific gravity rise to a height proportional to their

respective surface tensions. Another principle serving for measurement of surface tension is that of the *stalagmometer*. This is a dropping device (Fig. 21) from which a liquid is allowed to flow dropwise under standard conditions of temperature and pressure. Size of drops will be determined by the weight of the liquid and its surface tension. The number of drops required to deliver a given volume will vary inversely as the size of the drops. For a given standardized stalagmometer, then, the specific gravity of the liquid and the average number of drops, that fall during the delivery of 1 cc., give data sufficient to calculate the comparative surface tension of a liquid. With the stalagmometer, some liquid of known surface tension, expressed in absolute figures, is required as a substance to standardize the apparatus. Absolute figures may be obtained by the capillary method or by the Searle's balance. The latter instrument is a torsion balance shown in Fig. 22. The balance arm is so arranged that its movements twist a delicate thread of phosphor-bronze. A rectangular glass slide from the free end of the arm is permitted to just touch the surface of the liquid to be tested. The pull of the surface film of the liquid draws down the balance arm by an amount indicated upon a scale over which the point of the arm moves. The liquid is then removed and the slide dried. Weights are now placed in a small pan attached to the arm at the point from which the slide is suspended. When the weight added is just sufficient to pull down the pointer as much as surface tension had previously drawn it, the weight is recorded as equivalent to the surface tension. The latter acts along the line forming the boundary of the water in contact with glass; so that twice the length (l) of the slide plus twice the thickness (t) gives the total length of the line of film exerting tension. If M is the weight used to pull down the balance, gM is the total pull of gravity equivalent to the surface tension observed so that if l and t are expressed in centimeters,

$$\frac{gM}{2(l+t)} = \text{dynes of surface tension per centimeter of liquid surface.}$$

A dyne is the force which, acting on one gram during one second, can accelerate its velocity by 1 cc. per second; g is the acceleration constant of gravitation = 981 at sea level, 45°N. lat.

By such measurements the actual surface tensions of some solvents have been determined as follows:

Water.....	73 dynes per centimeter
Alcohol.....	22 dynes per centimeter
Ether.....	16 dynes per centimeter

These examples are sufficient to indicate that substances of different molecular constitution have widely differing surface tensions.

In any determination extraordinary care in the purification of the liquid and cleanliness of capillary tube, stalagmometer or glass ware used

in the balance method must be observed. Exceptionally minute traces of impurities, especially of fats, lipoids, waxes or soaps, will greatly diminish surface tension. Ordinary distilled water, for example, usually contains enough fatty material to distinctly lower its surface tension. Any substance in solution, as a rule, lowers surface tension at liquid-liquid interfaces while at liquid-gas interfaces all substances except inorganic salts tend to lower surface tension.

Concentration at the Surface.—Anything which lowers surface tension tends to be concentrated at the surface of its solution. In some cases this is capable of obvious demonstration since the concentrated substance may form a visible surface crust, or nearly rigid film. This has been shown with white of egg, and a number of other substances. It explains why beaten white of egg stiffens and changes appearance on standing. The albumin in it becomes actually coagulated at the surface of each bubble, as a result of surface concentration. Saponin bubbles similarly stiffen with great rapidity. For other substances a similar demonstration of surface condensation has been made by more complex physical methods.

The tendency to aggregate at the surface of a solution is directly proportional to the power of lowering surface tension. The latter is a variable quantity for different types of substances; but among the substances always found in protoplasm, soaps, lipoids and proteins are most effective, so that they are the substances, which must concentrate upon protoplasmic surfaces to form limiting membranes. To what extent other materials compose it we have little information. No artificial membranes composed of proteins alone, lipoids alone or combinations of them have shown exactly the same curious "selective" powers that living membranes possess.

The actual production of membranes upon protoplasmic surfaces appears to occur quite rapidly. The production of a vacuole in the substance of an amoeba gives an opportunity to observe membrane formation. Obviously the contents of the vacuole are not free to diffuse into protoplasm, so that a membrane must form as rapidly as the vacuole is produced. Again, if a pseudopodium of an amoeba is cut off, the living surface of the cytoplasm, thus exposed, very soon becomes normal as evidenced by the further formation of new pseudopodia. Similar behavior of the protoplasm of a number of kinds of cells has been observed after exposure of living surfaces by microdissections. The ingenious apparatus described by Kite is used for this purpose.

The Structure of Protoplasmic Membranes as Affected by Inorganic Ions.—An explanation of the physicochemical form of arrangement of the materials composing the membrane would help to explain membrane behavior. A mosaic structure has been suggested but this seems a fanciful descriptive term without satisfactory, experimentally established

significance. Described as a colloidal material of emulsion type, the membrane is perhaps better characterized in a way subject to experimental testing.

The so-called "antagonistic action" of salts upon living cells appears to throw some light upon the nature of limiting membranes. This action is demonstrated by the neutralization of the toxic effects of certain unphysiological concentrations of inorganic salts by the presence of the correct concentration of another salt. NaCl as it normally exists in sea water is, of course, not toxic to eggs of marine forms, such as those of *Fundulus* (the shore minnow) or of the sea urchin. But if such eggs are placed in a pure solution of sodium chloride of concentration comparable to sea water, they are rapidly killed. If, however, the solution is so prepared that for every 100 molecules of NaCl one or two molecules of CaCl₂ or of MgCl₂ are included, the solution is comparatively innocuous; so that fertilized eggs may develop in it to an advanced larval stage. This is one of scores of similar striking experiments that have been tried. Wheat will sprout, develop rootlets and go through the initial stages of growth in a watery solution containing 100 molecules of NaCl to one of CaCl₂; but in solutions of comparable strength, containing either sodium chloride or calcium chloride alone, practically no growth occurs.

Moreover, the work of Osterhout and others on the electrical conductivity of tissues has given another demonstration of this relationship between sodium and calcium. The electric current is conducted through solutions only by movements of ions formed by the electrolytic dissociation of acids, bases or salts. (See Chap. VI.) Since protoplasm is itself a solution, it may conduct the electric current only in so far as ions move through it. A mass of cells constituting a living structure will conduct the current more perfectly, that is, show less resistance, the more freely its membranes are permeable to ions. Osterhout studied the electrical resistance of the tissues of the marine kelp, *Laminaria*, and showed that when freshly prepared and still moistened with sea water it showed a certain resistance corresponding to a slight conductivity. When it was transferred to a pure sodium chloride solution, of concentration comparable to sea water, it had a much greater conductivity. This result indicates that sodium chloride increases the permeability of the cell membranes to ions. When, however, the kelp was again returned to sea water, after a not too long exposure to pure sodium chloride solution, the tissues tended to regain their original resistance. When the kelp was changed from sea water to pure calcium chloride solution, of concentration comparable to sea water, the electrical resistance was increased, indicating a decreased permeability; but this effect was also reversible; so that when replaced in sea water, the tissues regained normal permeability. An especially significant result was obtained when the material was placed in solutions containing both sodium chloride and calcium

chloride. Osterhout found that if the proportion was 100 molecules of the NaCl to one or two of CaCl₂, the conductivity showed no change from that observed in sea water. Any deviation from that proportion had an effect: Preponderance of sodium causing increased permeability (decreased resistance) and excess of calcium causing decreased permeability (increased resistance).

Such results help to explain the antagonistic action of sodium and calcium ions, showing how either one in sufficient excess might be toxic or fatal, while a proper balance of concentrations of both would annul the disastrous effects of each and produce suitable physiological conditions. It is obvious that free permeability of a living cell membrane or complete impermeability would be fatal and only a regulated semi-permeability is favorable to vital phenomena. If this favorable condition is dependant upon structure of protoplasmic membranes, then such structures must change sensitively in response to changes in relative abundance of sodium and calcium salts.

Factors Determining the Form of Emulsions.—How may ions, such as those of sodium and calcium, have an effect on cell permeability? Although an explanation is not yet complete, the following experiments with oil emulsions seem very significant. The effectiveness of soaps as emulsifiers has been discussed above. They exert this effect by means of their marked power to lower the surface tension of water, so that it no longer draws away from oil droplets, but prevents them from coalescing. Oil-water emulsions are of two types: (1) Oil droplets surrounded by water, as in cream; or (2) water droplets surrounded by oil, as in butter. Bancroft showed that if an oil was emulsified with water containing a sodium soap, emulsions of the first type (oil in water) resulted; but when calcium soaps were used, the other kind of emulsion (water in oil) was formed. While a sodium soap is more soluble in water than in oil, calcium soaps are more soluble in oil than in water; so that Bancroft interpreted his observations as indicating that sodium soaps lower the surface tension of water sufficiently to enable it to emulsify fat, while calcium soaps decrease the surface tension of oil so that it can emulsify water. Soap concentrates at the interface between water and oil, since it is drawn thither by surface tension; once present at the interface, it will become more concentrated in the surface layer of that one of the two liquids in which it is more soluble. That surface would then have a tension distinctly less than that of the liquid on the other side of the interface. The latter, obeying the principle of least or minimal surfaces will tend to draw itself into spheres which will be surrounded by the liquid whose surface tension was more markedly lowered. In other words the liquid with the higher surface tension will become the dispersed phase of an emulsoid colloid whose continuous phase is the liquid of lower surface tension (p. 78). When the proportion of calcium soap to sodium soap

is varied—as would be the case when a given quantity of oleic acid is treated with varying quantities of NaOH and CaCl₂—the emulsifying effects of the combination of soaps will depend upon whether sodium or calcium predominates. It should be possible to find a mixture containing such proportions of the two soaps that they counteract each other; so that neither type of emulsion could form, because the surfaces of oil and water have approximately equal surface tension. Any preponderance of sodium soap, as compared with the balanced proportion, would cause emulsion of oil in water; while preponderance of calcium soap would produce the other type of emulsion.

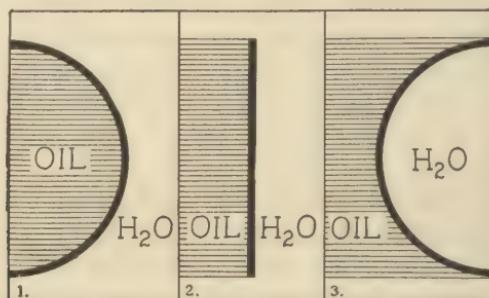


FIG. 23.—Diagram to illustrate the effect of surface tension in determining the form of an emulsion. Explanation in text. (From Clowes, *J. Phys. Chem.*).

Clowes, who undertook to test this possibility has illustrated the effect diagrammatically (Fig. 23). One of his experiments gave an especially striking demonstration of the principle. Portions of oil, containing a small amount of oleic acid for soap production, were shaken with equal volumes of water which, in the different tests, contained varying quantities of NaOH and CaCl₂. The results are shown in the following table:

$\frac{M}{10}$ Volume of NaOH used	Volume of $\frac{M}{10}$ CaCl ₂ used			
	0.25 cc.	0.5 cc.	0.75 cc.	1.0 cc.
Ce.				
1	0+	+++	+++	+++
2	000	0+	+++	+++
3	000	000	0+	+++
4	000	000	000	0+

10 cc. each of water and oil used in each case.

000 indicates oil dispersed in water.

0+ indicates critical or balance condition—no emulsion.

+++ indicates water dispersed in oil.

A photograph of the bottles containing the mixtures obtained in the experiment is shown in Fig. 24. The oil was colored red with Soudan

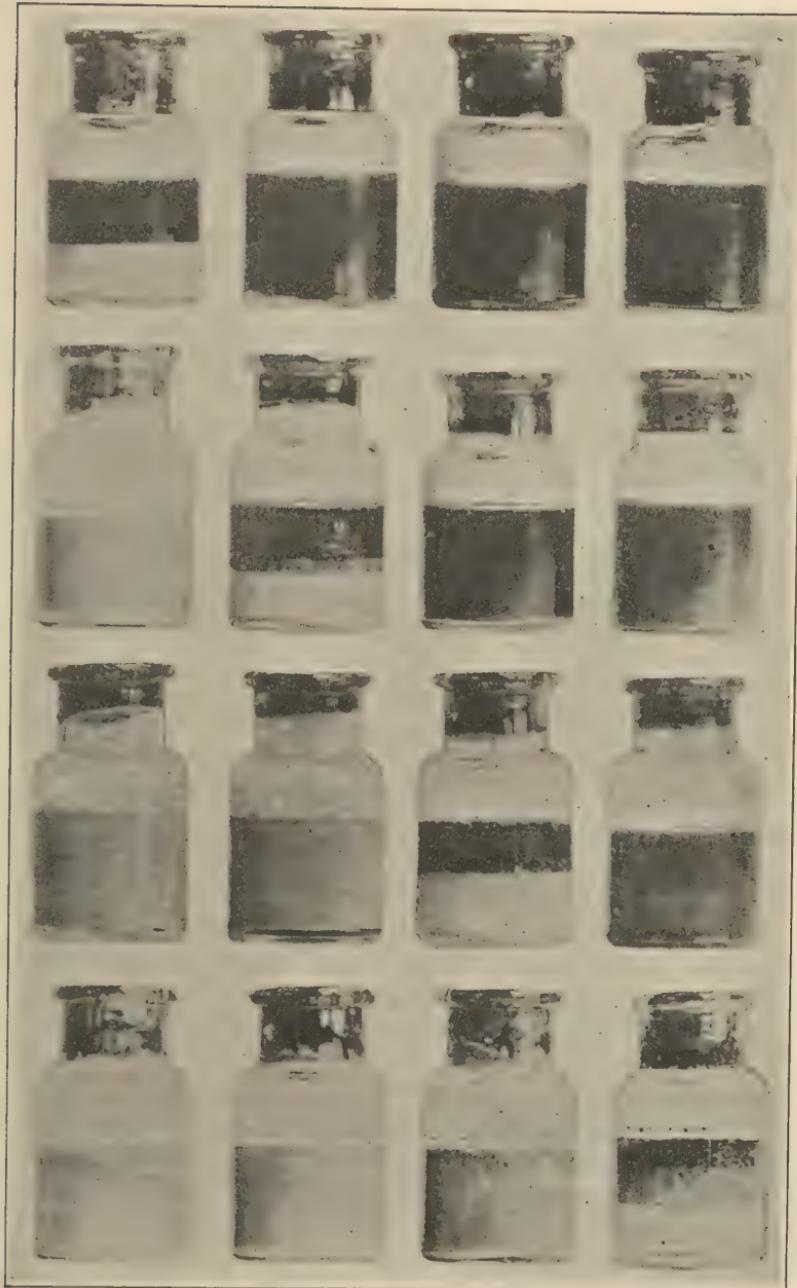


FIG. 24.—Oil and water mixtures showing the antagonistic action of certain ions in determining the form of the emulsion. Explanation in text. (From Clowes, *J. Phys. Chem.*)

III so that the butter-like emulsions with oil as the continuous (external) phase appear black in the picture. The cream-like emulsions having oil dispersed in water appear gray and the mixtures containing critical ratios of sodium to calcium show separate strata of oil and water.

This experiment indicates that, while a certain proportion of sodium and calcium in the presence of soap-forming fatty acids can produce one type of emulsion, a sufficient change in the relative abundance of these two may reverse the emulsion to the other type. Clowes was able to make this transformation by adding CaCl_2 to the cream-like emulsions obtained in the above experiment. He also observed the changes from one type to the other when a drop of emulsion in the field of the microscope came in contact with a solution containing an excess of either calcium or sodium salts.

The significance of the ability of an emulsion to be reversed lies in the fact that its permeability is thereby altered. Substances that are soluble in water may freely diffuse through an emulsion of the type having water as the continuous phase but only the so-called "lipoid-soluble substances" may pass through an emulsion in which fat is the continuous phase. Clowes has likened the first type of emulsion to islands surrounded by water and the second to lakes surrounded by land. Suppose the greater part of the semipermeable membrane of a cell were essentially an emulsion of watery substance dispersed in a fatty substance. It would be nearly impermeable to water-soluble substances like salts, sugars, and amino-acids. Normal cells are nearly impermeable to them. It would also be a very poor conductor of the electric current since it is the ions of salts in protoplasm and surrounding media which chiefly carry the electricity. The ions would be stopped at the membrane. This, too, is in accord with physiological observations. If the emulsion-like structure of the membrane contained soap, an increase in the abundance of sodium in the surrounding medium would increase its permeability to water soluble substances and correspondingly raise the electrical conductivity. Similarly, increased proportions of calcium would cause the reverse effects.

In the experiment as described, it will be noticed that the critical ratio of $\text{Na} : \text{Ca}$ is 4:1. In the experiments which show the antagonistic action of sodium and calcium ions on plant and animal cells the critical or balanced ratio is: $\text{Na} : \text{Ca} :: 100:1$ or 2. In a further series of experiments, employing a more delicate technique, Clowes was able to show that, in the presence of very dilute alkali, the critical or balancing ratio for effects of sodium and calcium on surface tensions between watery and oily phases is: $\text{Na} : \text{Ca} :: 100:1$ or 2. Clowes has drawn some interesting comparisons, in addition to those mentioned above, between his experiments and numerous biochemical observations on antagonistic action of salts. The reader is referred to the original papers for details.

For purposes of clarity, only sodium and calcium have been mentioned as influencing the equilibrium between fatty and watery phases so as to determine the form of emulsion. As a matter of fact, other ions are also concerned in determination of the actual result. Bancroft showed that di- and trivalent cations act in general like calcium while monovalent cations tend to behave like sodium. Clowes and others have studied the effects of a number of anions and cations upon emulsions. One may summarize such observations by saying that the effects of ions and other substances depend upon whether they tend to diminish the surface tension upon the lipoid side of the phase boundary or upon the watery side. Anything which disturbs the equilibrium at the oil-water interface can alter the condition of an emulsion. The antagonism of calcium and magnesium, under certain conditions, or of magnesium and sodium, under others, may be observed in physiological experiments and show parallel antagonisms in their effects upon emulsions. The most striking correspondence between disturbance of protoplasmic equilibrium and of emulsion equilibrium, however, is the one described as dependent upon the relative concentrations of calcium and sodium.

Does this justify us in the assumption that the thin limiting membrane of protoplasm is an emulsion with a continuous lipoid phase and a dispersed watery one, an emulsion so delicately balanced that disturbances in the ratio of various ions to each other may temporarily reverse it and produce a greater permeability to water soluble substances? The idea is a very attractive one and may well serve as a working hypothesis. Much remains, however, to be explained. Any complete theory of cell permeability must take into account the rôle of proteins in the behavior of the cell membrane. The covering of protoplasm is even more complex, physico-chemically, than is a lipoid emulsion. Moreover a satisfactory theory awaits knowledge of the actual circumstances, which, under normal conditions, permit the penetration of substances to which cells seem to be impermeable; for no one has yet discovered any *normally-acting* physiological mechanism that alters the effective proportions of calcium and sodium ions in its membranes.

Whatever future research may bring forth, the parallelism between the antagonistic action of ions in physiological effects, on the one hand, and in emulsion effects, on the other, is too close to be without significance. The behavior of fats and lipoids in emulsions, such as we believe are present at cell surfaces, promises a solution of the problem of cell permeability.

The Non-diffusible Condition of Cell Constituents.—What has been said concerning the membranes of protoplasm should not be construed as meaning that they alone determine what substances shall be enabled to enter living cells and what shall be retained within them or what be ejected. Many facts, to be encountered in later chapters, show that the

cell, acting as a whole, may be the determining factor in certain cases. It will be recalled that in discussing proteins as amphoteric colloids (p. 55) their remarkable powers of combining in different ways with many kinds of substances was explained. Being themselves in a colloidal state, proteins must hold the things that are combined with them in a non-diffusible condition. Other cell colloids, for example lecithin, might similarly function. Such a process offers an explanation of the protoplasmic power to retain certain things without possessing a limiting membrane always impermeable to them. A notable instance is the case of potassium retention. In a great number of plant and animal cells, potassium is much more concentrated than in the surrounding medium. In blood corpuscles, it is twenty times as concentrated as in the surrounding plasma. Similar though less striking figures could be given for other cells. The accumulations of potassium in seaweed, such as the giant kelp of the California coast, are so great as to make feasible its use as a source of commercial potash. The very name potash refers, of course, to the time-honored practice of utilizing wood ashes as an alkali on account of the K_2CO_3 in them. This is due to the accumulations of potassium in the hard, cellulose parts of plants. The potassium must at some time penetrate cells, so that the postulation of a limiting membrane absolutely and continually impermeable to it is obviously absurd. From all our observations on the distribution of inorganic ions on the two sides of a membrane permeable to them, we know that diffusion of ions through the membrane continues until the concentration is equal on both sides. The comparatively enormous accumulations of potassium are not retained by being merely dammed up; but supposing it is not present as an inorganic ion, but is, in part at least, held in a non-diffusible colloidal combination, its retention by the cell is not surprising. There is plentiful evidence that various other substances, notably amino-acids and sugars, are held within the cell in non-diffusible combinations similar to those of potassium. The manner of their penetration into cells, the mechanism by which they are originally absorbed, is still highly problematical, as suggested in the preceding paragraph. Some of the evidence that may be further brought to bear upon it will be discussed later. (Chap. X.)

Other Functions of Lipoids.—There are other functions of lipoids of a more specific character. The phospholipin, *cephalin*, or some substance associated with it has been shown to have a marked accelerating effect on clotting of blood. It is present, apparently, in every kind of animal cell. All dead, disintegrating cells or extracts of dead animal tissues markedly accelerate blood clotting. Substances derived from some such source are, indeed, absolutely requisite for normal blood clotting and are usually supplied by dying structures in shed blood or by injured cells of a wound or by both. The physiological value of blood coagulation as a check to excessive bleeding is obvious; so that the importance of cephalin,

in so far as it enters into the process, needs no emphasis. The manner of its action will be described in connection with the physiology of the blood. The marked effect of the phospholipin, *tethelin*, upon growth has already been mentioned (p. 69).

Lipoids may also be useful in furthering the utilization of neutral fats. Analysis of plasma, the fluid part of blood, and of the corpuscles and other cells has shown a marked tendency of the body to maintain a certain uniform relation between the quantities of neutral fat, cholesterol esters and lecithin in the blood fluid and body cells. This uniformity of the proportions between them has led to the hypothesis that fats must be first synthesized into lecithin or into cholesterol esters before utilization in the body.

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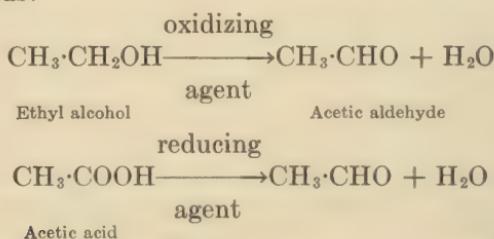
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CHAPTER IV

THE CARBOHYDRATES: THEIR CHEMISTRY AND THEIR RÔLE IN LIFE PROCESSES

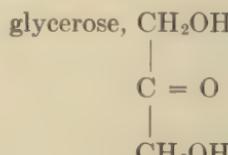
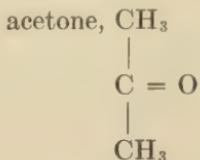
Definition.—Carbohydrates are roughly defined as the starches and sugars and related substances, since starches and sugars are the chief members of the group. The word, carbohydrate, was chosen under the impression that all carbohydrates were carbon compounds containing hydrogen and oxygen in the same proportion as in water—"hydrates of carbon." This is true for nearly all carbohydrates, as is indicated by the formula of a typical representative, glucose, $C_6H_{12}O_6$. But many substances, other than carbohydrates, have a composition answering to the same description (acetic acid, $C_2H_4O_2$); while carbohydrates without this proportion are known (rhamnose, $C_6H_{12}O_5$). The only satisfactory definition of the carbohydrates is based on their molecular structure. Thus considered, they are *the aldehyde or ketone derivatives of the polyhydric alcohols*.

In explanation of this definition, consider first the aldehydes. They are compounds whose characteristic atomic group is $\begin{array}{c} \text{O} \\ \diagdown \\ \text{C} \\ \diagup \\ \text{H} \end{array}$, also written, $-\text{CHO}$; examples: formaldehyde, $\text{H}\cdot\text{CHO}$, acetic aldehyde, CH_3CHO , etc. They are produced either by oxidation of an alcohol or by reduction of an acid. Thus:

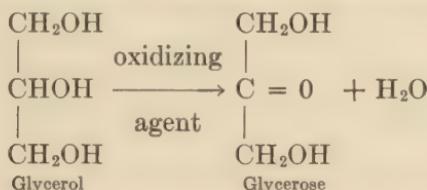


By progressive oxidation an alcohol may be oxidized to an aldehyde and then to an acid. Such stages may be recognized as occurring during the oxidation of substances in living organisms.

Next consider the ketones. They are compounds in which the typical atomic grouping is: $-\text{C}-\text{CO}-\text{C}-$; examples:

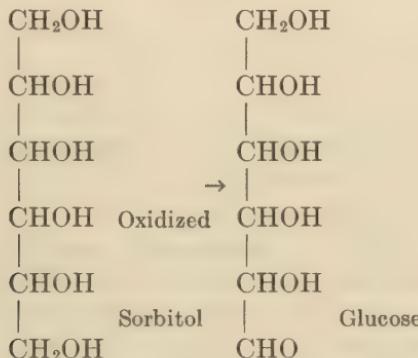


They too may be formed by special types of oxidation of alcohol. Thus:

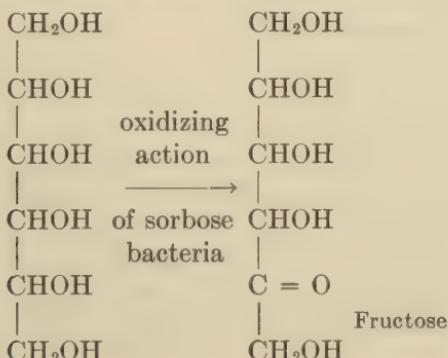


The term polyhydric alcohols has been explained in connection with glycerol (Chap. III) as referring to alcohols with more than one —C—OH group in the molecule.

That the carbohydrates are derivatives of such alcohols is seen from certain methods for the production of typical ones. An alcohol, known as sorbitol, found in certain plants, for example, in the sap of the mountain ash, and also produced by artificial synthesis, may be oxidized to form the typical carbohydrate, glucose.



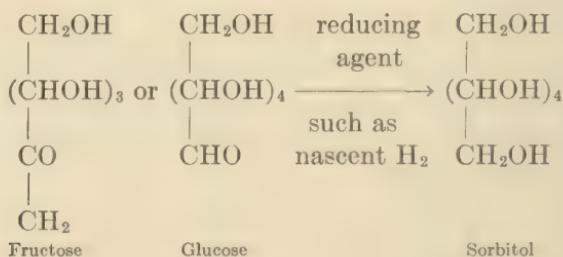
Thus a hexahydric alcohol is changed to a compound having five alcohol groups and one aldehyde group. By a different type of oxidation, however, sorbitol is changed to another sugar, fructose.



In this case, hexahydric alcohol is changed to a compound having five alcohol groups and one —CO group, so placed as to give the ketone struc-

ture. Glucose and fructose are the most widespread and abundant carbohydrates in nature. The other less abundant sugars have similar structures and as all carbohydrates, other than sugars, are merely polymerizations (condensations) of two or more molecules of sugar, the entire group is properly defined as aldehyde and ketone derivatives of polyhydric alcohols.

That the carbohydrates are derivatives of alcohols is further indicated by the reduction of sugars to polyhydric alcohols. Both glucose and fructose, for example, reduce to sorbitol. The reaction is represented thus:



Other sugars similarly reduce to their corresponding polyhydric alcohols and carbohydrates, other than sugars hydrolyze to form sugars that reduce to polyhydric alcohols. The relationship of carbohydrates to polyhydric alcohols is, then, a general one.

In the formulas given for glucose and fructose, the location of the aldehyde or ketone group is definitely shown. The proof of the position has been obtained, in each case, from different kinds of evidence. Oxidation reactions are perhaps the most convincing. Glucose, on mild oxidation, changes to gluconic acid, $\text{CH}_2\text{OH}\cdot(\text{CHOH})_4\cdot\text{COOH}$, and since the aldehyde group is known to oxidize to a carboxyl group much more readily than do alcohol groups, the known structure of gluconic acid (carboxyl group at the end of the chain of six carbon atoms) proves that glucose has its aldehyde group at the terminal position. A corresponding oxidation of fructose proves that its $-\text{C} = \text{O}$ group lies in the fifth position of the carbon chain. Lack of space forbids a description of the many and varied methods used in establishing the exact structure of these and other sugars. All of the known natural sugars and many related synthetic ones have been studied thoroughly enough to establish their structure thoroughly. Much of this work we owe to Emil Fischer who was a leading pioneer in this field, as in that of protein and amino-acid investigations. Details may be found in treatises on Organic Chemistry.

Carbohydrate Classification.—Carbohydrates are grouped according to molecular structure; so that their classification gives an insight into the chemical nature of the different carbohydrates. The main subdivisions are made in accord with the number of typical groups occurring in the

molecule. *Monosaccharides* have one sugar group; *disaccharides*, two, etc. In the *polysaccharides* the number of constituent groups has not been determined with certainty.

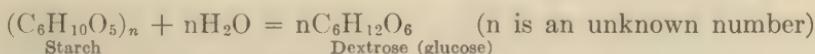
The monosaccharides are subdivided according to the number of oxygen atoms in the molecule. Thus there are recognized: Trioses, $C_3H_6O_3$, tetroses, $C_4H_8O_4$, pentoses, $C_5H_{10}O_5$ and hexoses, $C_6H_{12}O_6$ which occur in nature and higher ones up to nonoses, $C_9H_{18}O_9$, which have been prepared artificially. Only the *pentoses* and *hexoses* are frequently found in plant and animal structures. They are the only monosaccharides occurring in abundance and are the most important food carbohydrates. Both pentoses and hexoses are found to be of the two types: Aldoses, (aldehyde derivatives) and ketoses, (ketone derivatives).

Disaccharides are combinations of two molecules of the same or of different monosaccharides with the elimination of one molecule of water. Not many different disaccharides have been discovered and each one is known by a special name.

Trisaccharides and tetrasaccharides are still less numerous, only two of each having been described.

Polysaccharides are a very large group and occur abundantly. They form the chief constituents of the hard, cellulose parts of plants. In addition to cellulose materials, the group includes the starches, the vegetable gums and dextrans and many other constituents of plants and animals. It is convenient, therefore, to subdivide them according to their structure. As each one, with a few possible exceptions, consists of a polymer of one monosaccharide, they may be named in accordance with the sugar serving as a unit building stone. Starch, built up of dextrose (another name for glucose) molecules is called a dextran. Each polysaccharide may be named by adding the termination "an" to the root word for its constituent monosaccharide.

The relation of the more complex carbohydrates to the monosaccharides is revealed by the results of hydrolysis. All the agents of hydrolysis, that affect the proteins, have their corresponding representatives that affect the carbohydrates, namely: Water, superheated steam, boiling acids, boiling alkali (which causes oxidation in addition to hydrolysis) and certain carbohydrate-splitting enzymes that are found in plants and animals. Monosaccharides cannot be altered by hydrolytic agents without losing their carbohydrate character. The general nature of the hydrolytic reaction is indicated by the following examples:



The following classification shows the structural relationships of the well-known natural carbohydrates, and their chief sources.

Group	Sub-group	Type	Name	Remarks
I. Monosaccharides	Bioses, $C_2H_4O_2$	Aldose	Glycolaldehyde	Not of great physiological significance.
	Trioses, $C_3H_6O_3$	Aldoses	Glycerose	
	Ketoses		Dioxyacetone	
	Tetroses, $C_4H_8O_4$	Aldoses	Erythrose	
		Ketoses	d-Erythrulose	
	Pentoses, $C_5H_{10}O_5$	Aldoses	Arabinose	From hydrolysis of cherry and other tree gums.
			Xylose	
			Ribose	
		Ketoses	L-Arabinulose	From hydrolysis of nucleoproteins.
		Aldoses	Dextrose or glucose	
II. Disaccharides, $C_{12}H_{22}O_{11}$.	Hexoses $C_6H_{12}O_6$	Aldoses	Galactose	In blood and other animal fluids, plant juices, etc. and from hydrolysis of many polysaccharides and other biological compounds. The most abundant and widespread of all carbohydrates.
			Mannose	From hydrolysis of lactose and certain other polysaccharides.
		Ketoses	Levulose, or fructose	From hydrolysis of rare polysaccharides.
			Sorbose	In fruit juices and from hydrolysis of saccharose, inulin and other carbohydrates.
				From Sorbitol by a specific enzyme.

II. Disaccharides, $C_{12}H_{22}O_{11}$.

Saccharose, in all sweet tasting plant juices. Hydrolyzes into dextrose + levulose.

Maltose, in malted grains, from partial hydrolysis of starch. Hydrolyzes into dextrose + dextrose.

Isomaltose, from action of certain enzymes on maltose.

Lactose, in milk, is a specific product of mammary gland. Hydrolyzes into dextrose + galactose.

Isolactose, from action of certain enzymes on lactose.

Melibiose, from partial hydrolysis of Raffinose. Hydrolyzes into dextrose + galactose.

III. Trisaccharides, $C_{18}H_{32}O_{18}$.

Raffinose (melitose) in molasses and a number of plant juices, in bacteria and fungi. Hydrolyzes into levulose and melibiose.

Melibiose, in twigs of the larch (*Pinus larix*).IV. Tetrasaccharides, $C_{24}H_{42}O_{21}$.

Lupeose, in peas. Hydrolyzes into two molecules of galactose one of glucose and one of levulose.

V. Polysaccharides.

(A) Pentosans, condensations of pentoses.

1. Arabans, in gum arabic, cherry gum, etc. Hydrolyze into arabinose.

2. Xylans, in hemicelluloses of wood and other plant tissues. Hydrolyze into xylose.

(B) Hexosans, condensations of hexoses.

1. Dextrans, hydrolyze into dextrose.

Starch, in the leaves and storage parts of nearly all plants.

Cellulose, in wood pulp, cotton fibers, etc.

Dextrins, in vegetable mucilages and from partial hydrolysis of starch.

Glycogen, in nearly all animal tissues.

Lichenin, in various lichens.

2. Levulans, hydrolyze into levulose.

Inulin, in storage parts of *Compositæ*.

3. Galactans, hydrolyze into galactose.

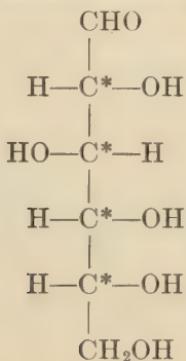
Agar-agar from various seaweeds, (may also contain arabinose in combination with galactose).

(C) Polysaccharides of unknown composition.

Many of the vegetable gums, the pectoses and pectins, hemicellulose constituents, etc., etc. Hydrolyze into galactose and arabinose in some cases but other hexoses and pentoses may also be present.

All the group of polysaccharides exist in nature as complex colloidal combinations with each other and with inorganic substances. Many so-called celluloses and all the hemicelluloses yield, on hydrolysis, both hexoses and pentoses as well as phosphates and other inorganic constituents. No way has been found to isolate pure individual polysaccharides from many of the natural complexes; so that the existence of some of them as distinct compounds is hypothetical. As actually found in plant tissues, some of them are probably phosphates, others are calcium or potassium combinations and all of them are in complex form. Glycogen and certain specimens of starch are the only polysaccharides that have been isolated in what is probably chemical purity. While both pentoses and hexoses occur in polysaccharide compounds; yet so far as is known, only hexoses are built into di-, tri- and tetrasaccharides.

The Stereoisomerism of the Sugars.—As already explained (Chap. II), stereoisomerism is due to the presence of asymmetric C atoms in the molecule. The formula for glucose shows four of them.



It rotates the plane of polarized light to the right and has therefore been named dextrose. It possesses an optical mate showing an equal levorotation; but the latter is unknown in nature and is prepared only by artificial synthesis. In fact, all synthetic sugars, like other compounds artificially produced in imitation of natural ones, tend to appear in racemic form; that is, they are mixtures of equal parts of optical mates. All natural sugars, however, like all other asymmetric, natural compounds, are optically active. This, it will be recalled, is supposedly due to the fact that they are produced from optically active materials by the optically active reagents of protoplasm, the enzymes.

The Aldo-hexoses.—It would seem, at first sight, somewhat strange that there are three different sugars all proved to be aldo-hexoses: Dextrose, galactose and mannose. The same abbreviated structural formula may stand for each of them: $\text{CHO}\cdot(\text{CHOH})_4\cdot\text{CH}_2\text{OH}$. But in the various sugar reactions, to be described presently, they all give different results. Moreover, each has its own characteristic specific

rotatory power. They are by no means identical compounds. Their behavior in the living organism is not the same. It is found, for example, that the quantity of each that may be injected into the blood of an animal, without being excreted into urine, is different; so that the rate of utilization in the body is characteristic for each of them. Dextrose is utilized best, mannose less rapidly and galactose only very slowly. Dextrose appears free in blood and combined in various ways in animal tissues; galactose occurs in only two combinations, cerebrosides and lactose; while mannose is unknown in the animal world. Similar specificity of the usefulness of the different sugars is found in plant physiology. The chemical laboratories, that we call living cells, make very sharp distinctions between these three, nearly identical sugars. What is the essential difference between them?

Recalling the characteristics of the asymmetric carbon atom, one can explain the nature of this isomerism. Any such atom may exist with either of two arrangements around it. One turns polarized light to the right and the other to the left. Each arrangement confers specific properties to the molecule. In a grouping containing four asymmetric carbons, each one may have either of its two possible arrangements. Mathematically, one may prove that 16 combinations of 4 asymmetric carbon atoms are possible. Indeed, for any compound, the number of stereoisomers is equal to 2^n where n is the number of asymmetric carbon atoms in its molecule. The following scheme will show the 16 possibilities for an aldo-hexose. The + sign is supposed to represent an asymmetric carbon-containing group that causes a dextrorotatory optical effect; the - sign stands for one producing levorotation.

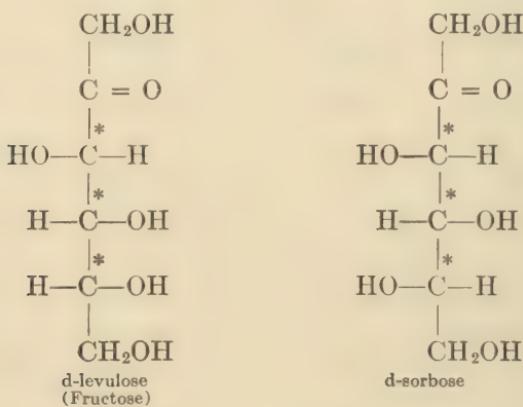
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
+	+	+	+	-	-	-	-	-	+	+	-	+	-	+	-
+	+	+	-	-	-	-	+	+	-	-	+	+	-	-	+
+	+	-	-	-	-	+	+	-	+	-	+	-	+	+	-
+	-	-	-	-	+	+	+	-	-	-	+	-	+	+	-

It will be seen that each carbon appears as a + eight times and as a - eight times. All these combinations are possible and no others. It might seem as though certain ones, for example, numbers 3 and 7, were identical; but such is not the fact because the terminal groups of aldo-hexose; (-CHO and -CH₂OH), are different so that they have specific and different effects upon the carbon atoms directly attached to them. All of these 16 different sugars can be prepared by appropriate syntheses and in each case, the optical arrangement of each grouping in the molecule has been established. In the representations shown below, the true spacial arrangements can only be indicated in two dimensions; so that we have a merely conventionalized presentation or the fact that they all are grouped in optical pairs. Each one of a pair is the mirror image of the other.

CHO	CHO	CHO	CHO
H—C—OH	HO—C—H	HO—C—H	H—C—OH
HO—C—H	H—C—OH	HO—C—H	H—C—OH
H—C—OH	HO—C—H	H—C—OH	HO—C—H
CH ₂ OH	CH ₂ OH	CH ₂ OH	CH ₂ OH
-d-GLUCOSE (Dextrose)	l-glucose	d-MANNOSE	l-mannose
CHO	CHO	CHO	CHO
H—C—OH	HO—C—H	HO—C—H	H—C—OH
HO—C—H	H—C—OH	HO—C—H	H—C—OH
HO—C—H	H—C—OH	HO—C—H	H—C—OH
H—C—OH	HO—C—H	H—C—OH	HO—C—H
CH ₂ OH	CH ₂ OH	CH ₂ OH	CH ₂ OH
d-GALACTOSE	l-galactose	d-talose	l-talose
CHO	CHO	CHO	CHO
H—C—OH	HO—C—H	HO—C—H	H—C—OH
HO—C—H	H—C—OH	HO—C—H	H—C—OH
H—C—OH	HO—C—H	H—C—OH	HO—C—H
HO—C—H	H—C—OH	HO—C—H	H—C—OH
CH ₂ OH	CH ₂ OH	CH ₂ OH	CH ₂ OH
d-idose	l-idose	d-galose	l-galose
CHO	CHO	CHO	CHO
H—C—OH	HO—C—H	HO—C—H	H—C—OH
H—C—OH	HO—C—H	H—C—OH	HO—C—H
H—C—OH	HO—C—H	H—C—OH	HO—C—H
H—C—OH	HO—C—H	H—C—OH	HO—C—H
CH ₂ OH	CH ₂ OH	CH ₂ OH	CH ₂ OH
d-allose	l-allose	d-altrose	l-altrose

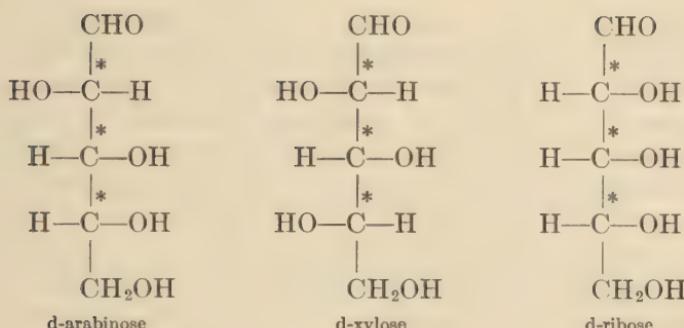
The three occurring naturally are indicated in capitals. So far as is known, the other thirteen are obtainable only by artificial synthesis.

The Keto-hexoses.—A similar tabulation might be made to show the possible keto-hexoses. As there are three asymmetric carbon atoms, eight (2^3) modifications might exist. Two of them have been found in nature and a few of the others have been synthesized. The stereo arrangement of the natural ones is shown thus:



The three asymmetric C atoms of levulose have the same arrangement as the corresponding ones in glucose. Levulose, the very common sugar also known as fructose or fruit sugar, is written d-levulose although it turns the plane of polarized light to the left, as the name levulose indicates. The prefix d- is used, in accordance with the suggestion of Emil Fischer, to call attention to the fact, established by ample evidence, that three of its asymmetric carbon atoms have exactly the same arrangement as d-glucose (dextrose). A glance at the formula for the aldo-sugar, d-mannose, shows that it, too, is very similar to glucose. It is significant that of the five natural hexoses three have the dextrose type of asymmetry and show a corresponding similarity in their usefulness in animal bodies and as food for microorganisms. Dextrose and levulose are by far the most abundant and widely distributed of all the sugars, so that the similarity of their stereo arrangement is supposed to indicate that the living substance, which first produced carbohydrate, was of such asymmetry that it tended to produce the dextrose type only. Organisms, appearing in the course of evolution, inherited the same tendency.

The Pentoses.—Among the pentoses, the same stereoisomerism exists. With three asymmetric carbon atoms, eight aldopentoses are possible. Three of them, d-arabinose, d-xylose and d-ribose, have been found in nature. The others have been produced synthetically. The natural ones are represented as follows:

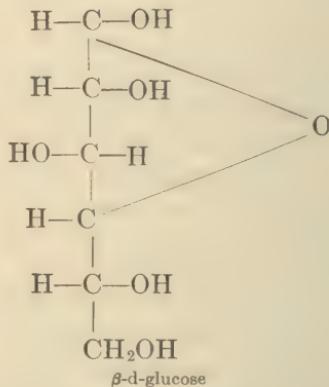
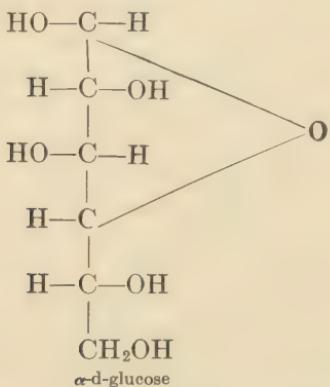


Arabinose, like mannose and levulose, is built on the same plan as dextrose. Because of this fact, the appearance of arabinose in human urine has received a tentative explanation. The symptom is known as *pentosuria* and may occur in seemingly healthy persons. Now, although ribose is found in animal tissues, where it occurs as a group in certain complex molecules, arabinose is not found in animal structures. Arabinose would seem to be formed, then, from its close relative, dextrose, which, ordinarily oxidized in animals to CO_2 and H_2O , might encounter some peculiarity in the metabolism that causes pentosuria and be oxidized to arabinose. This seems all the more probable, since the identical transformation may be obtained by suitable oxidation of dextrose in the test tube. But strangely enough, the particular form of arabinose, found in some of the few cases of pentosuria on record, is racemic; so that in this instance the general rule, that living matter produces only one of a pair of optical isomers, seems to fail.

Of keto-pentoses, little is known concerning their distribution in nature or their systematic stereochemistry.

Intramolecular Changes, Tautomerism.—Stereoisomerism of the sugars is further complicated by their ability to form intramolecular rearrangements (tautomerism). Glucose, for example, probably occurs in several tautomeric forms. Two of these are called lactone forms and two are called ethylene-oxide forms. The lactone forms are present in ordinary glucose solutions. Their presence is shown by the phenomenon of *mutarotation*, also called taurorotation, birotation or multirotation. It is observed in the following way. Pure anhydrous glucose (dextrose), if dissolved in water and observed in the polariscope at once, shows approximately twice the rotatory power that is really characteristic. If continuously observed, the rotation is found to gradually fall off until, after some 24 hours at room temperature, it comes to an equilibrium. The specific rotatory power is then +52.5. The explanation of this mutarotation was found in the presence of more than one kind of dextrose and the partial change of one into the other. One form may be crystallized out alone from 70 per cent alcohol at room temperature. It is

called α -d-glucose and has a specific rotation of approximately +110. The other form has been crystallized separately from hot water. It is called β -d-glucose and shows a specific rotation of +19. Either one of these, if dissolved in water and made faintly alkaline with ammonia, rapidly changes to a specific rotation of +52.5. The same changes occur slowly in a neutral solution. It is impossible to have two dextrorotatory modifications of dextrose unless a fifth asymmetric carbon, in addition to the four already described, is present. Because of this fact and in consideration of the behavior of certain derivatives of the two modifications, glucose is believed to exist in such form that its carbon atom in the aldehyde group is rendered asymmetric by union, through oxygen, with another carbon. The resulting lactone structure in its two optical forms is represented thus:



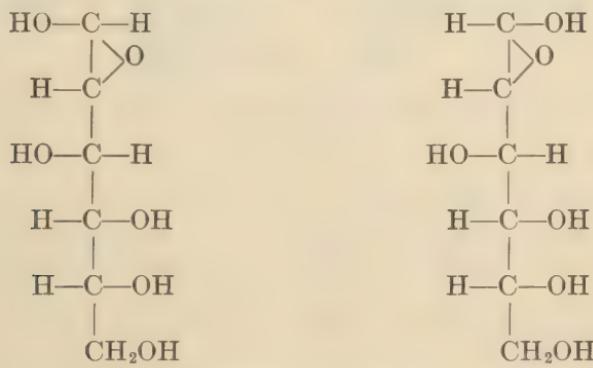
Ordinary glucose at equilibrium in solution is a mixture containing both these forms. No aldehyde group is present in either, yet glucose behaves like an aldehyde. Many of its test reactions, for example, give clear indications of the presence of an aldehyde group, capable of ready oxidation. But glucose oxidizes slowly; so that, while boiling in a dilute alkali, it gradually disappears. The rate of its disappearance is an indication that it is not all present as aldehyde at one time. The inference is that glucose, though largely in the α - and β -lactone forms, does contain in its solutions a small amount of the aldo-form. As fast as the latter oxidizes, more of it is produced.

The theory of the existence of these modifications of glucose has furnished the key to an explanation of many puzzling peculiarities of its behavior in vital processes: (1) Glucose enters into the synthesis of four distinctly different disaccharides (α - and β -maltose and α - and β -isomaltose) according to how the α - and β -glucose forms are joined together; (2) glucose, although the most readily oxidized of all cellular constituents, may still be held in the blood without suffering oxidation; (3) glucose enters into the formation of numerous derivatives, the gluco-

sides, so as to produce different stereoisomeric modifications, the α - and β -glucosides, each specific in its physiological behavior. Appreciation of the unquestioned physiological importance of glucose depends largely on knowledge of its peculiar asymmetry.

D-galactose, d-mannose, d-levulose, and some of the disaccharides show the phenomenon of mutarotation so that they too are probably able to assume the lactone formation in the molecule. Inasmuch as these are all naturally occurring sugars, the significance attached to the changeability of the dextrose structure applies to them as well.

The occurrence of ethylene oxide forms of glucose has been postulated to account for the fact that this sugar can be prepared in such a state that it is comparatively unstable and oxidizes much more readily than ordinary glucose does. By a study of some of the derivative compounds of this unstable form, Fischer and others have furnished evidence to show that glucose can change into what is called its γ form, which probably is a mixture of these two ethylene oxide arrangements:



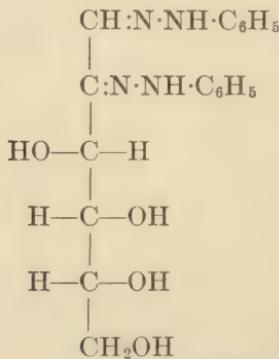
γ Form of Glucose

Hewitt and Pryde have shown that when glucose solutions are introduced into a tied off loop of a living intestine and allowed to remain there during about an hour, the glucose is so changed in rotatory power and other properties as to indicate that some of the γ form has been produced. This does not occur in a dead intestine. The comparative susceptibility of the γ form to oxidation suggests that it may be the form in which glucose is actually used in the animal body.

If glucose occurs, as seems very probable, in no less than five different arrangements of atoms within its molecules, it is a remarkably mutable substance. It remains for future investigation to show how this changeability is related to the undoubted fact that glucose is an indispensable nutrient for all animals.

The Typical Test Reactions of the Sugars.—The *Osazone Reaction*, has been, historically, of great importance in sugar chemistry. It was the

first method, discovered by Emil Fischer, for the satisfactory separation and identification of the sugars. Practically, it is of proved usefulness in qualitative detection of sugars in urine and other biological fluids and in food analysis. In principle, the reaction consists in the union of the sugar molecule with two of phenylhydrazine ($C_6H_5\cdot NH\cdot NH_2$). In practice, a number of modifications of method are available. One of the best methods is to treat the sugar solution with phenylhydrazine hydrochloride ($C_6H_5\cdot NH_2\cdot NH_2\cdot HCl$) and sodium acetate ($NaOOC\cdot CH_3$). The reagents are mixed dry by grinding in a mortar two parts, by weight, of phenylhydrazine hydrochloride and three parts of sodium acetate and are then dissolved in water or in the sugar solution. When dissolved they react to form acetic acid, sodium chloride and free phenylhydrazine which combines with the sugar in two stages: first, a phenylhydrazone having one phenylhydrazine group attached to sugar and constituting a soluble compound; second, an osazone having two phenylhydrazine groups attached to sugar and showing a tendency to crystallize out from solution. The osazone from a hexose may be represented thus:



Dextrosazone (from dextrose)
Also called glucosazone.

The osazones are glistening, bright yellow crystals of microscopic size and of a form which is characteristic for each different kind of osazone. The various ones are identified not only by crystalline form (Fig. 25) but also by their characteristic solubilities and the melting points of pure dried preparations. In the formula for dextrosazone, it is seen that the two phenylhydrazine residues are so attached to the sugar molecule as to completely mask the two groups which serve as the only distinguishing features of d-dextrose, d-mannose and d-levulose. The osazones from these sugars are absolutely identical and this fact was the first thing to call the biochemists' attention to the general uniformity of the structural plan (see above) of the naturally abundant carbohydrates. Pentoses as well as hexoses and certain disaccharides give osazones. The trisaccharides and other polysaccharides do not respond to the reaction.

The *polarization test*, for detection and measurement of the optical activity of sugars, is made in accordance with the method previously described (p. 48). Its fundamental value, in attempts to reach a theoretical explanation of the nature of isomeric sugar structure, has already been shown. Its practical value is due to the ease with which quantitative determinations of sugar may be made in the polariscope. In any

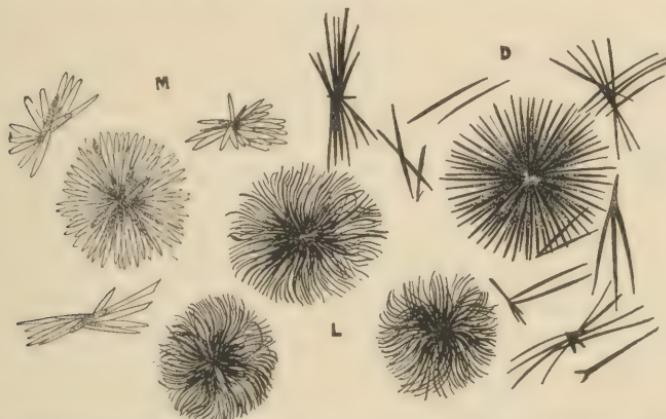
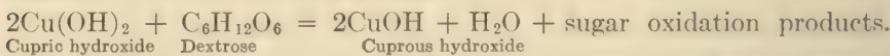


FIG. 25.—Microscopic appearance of osazone crystals. *M*, maltosazone. *L*, lactosazone. *D*, dextrosazone.

solution containing sugar, or sugars, of known kind and free from other optically active substances, the amount of sugar may be very closely estimated from the measurement of rotation observed. Recalling the formula for specific rotation, $(\alpha)_D = \frac{\alpha}{w \cdot l}$, it will be seen that if $(\alpha)_D$ has been previously determined, the equation in the form, $w = \frac{\alpha}{(\alpha)_D \cdot l}$ enables one to compute w (weight of substance in 1 cc. of solution), since l (length of tube) is known and α may be measured. The polariscope is used for rapid determination of sugar in urine and other biological fluids and in the analysis of foods and food products, including the detection of sugar adulterations. Substitution of glucose for the more expensive saccharose is detected in this way. A polariscope especially designed for sugar work is called a saccharimeter. Details of its use in commercial sugar chemistry may be found in practical treatises on the subject. Although all naturally occurring carbohydrates are optically active, the use of the polariscope in practical analyses of polysaccharides is somewhat limited, since many of them, owing to their colloidal character, do not give clear solutions sufficiently transparent for polariscope observation. It is notable, however, that the more complex the carbohydrate the higher its specific rotation tends to be. This will be apparent on consultation of the table on page 114 and is to be expected; since the more complex the

carbohydrate, the larger will be the number of carbon atoms in each molecule.

The so-called *reduction tests* for sugars are numerous. Most of these tests employ the same principle: The reduction of a metallic hydroxide in alkaline solution to an oxide or a free metal. This reaction is due to the tendency of sugars to take away oxygen, becoming themselves simultaneously oxidized to various products. In *Fehling's*¹ and *Benedict's*² tests copper sulphate is in a solution made alkaline in the first with potassium hydroxide and in the second with sodium carbonate. To prevent the precipitation of copper hydroxide rochelle salts are added to Fehling's solution and sodium citrate to Benedict's. Either of these reagents boiled with a sugar, containing a free aldehyde or ketone group, gives a red precipitate of cuprous oxide. *Nylander's* is a somewhat similar test. The solution used contains bismuth nitrate, rochelle salts and potassium hydroxide and after heating with sugar a black precipitate of metallic bismuth forms. Similar tests, employing metallic compounds, notably mercury and silver, have been devised. Benedict's reaction has proved to be especially useful in qualitative detection of sugar and, suitably modified, has become the basis of quantitative methods for sugar estimation. The sort of reaction occurring in reduction tests is typified by copper reduction in Fehling's test:



Cuprous hydroxide appears as a yellow precipitate but on further heating changes to red cuprous oxide.



The products of sugar oxidation as formed in the reaction are numerous and vary with slight changes of conditions. Most of them are organic acids which, of course, combine with the alkali present in the solution. The acid oxidation products of sugars, some of them formed in these reac-

¹ Fehling's solution is made as follows:

Solution A.

34.64 g. of crystalline copper sulphate, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, are dissolved in water and made up to 500 cc.

Solution B.

125 g. KOH and

173 g. Rochelle Salts, sodium potassium tartrate, $\text{NaK}(\text{OOC})_2 \cdot \text{C}_2\text{H}_4\text{O}_2$ are dissolved in water and made up to 500 cc.

Equal parts of solutions A and B are mixed and heated to boiling before adding the solution to be tested for sugar.

² Benedict's solution is prepared as follows:

85 g. sodium citrate, $\text{NaC}_6\text{H}_5\text{O}_7 \cdot 11\text{H}_2\text{O}$ and

50 g. anhydrous Na_2CO_3 are dissolved in 400 cc. water. 8.5 g. copper sulphate dissolved in 50 cc. hot water are then added slowly with stirring. The resulting solution, filtered if necessary, may be made up to 500 cc.

tions, others produced in animal oxidations of sugar, include: Gluconic, saccharic, tartaric, lactic, pyruvic, tartronic, succinic, malic, oxalic and carbonic acids.

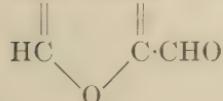
Reducing sugars may also show their effects by reducing copper acetate in acetic acid solution. This principle is utilized in *Barfoed's*¹ test to which disaccharides respond slowly and only after a long period of boiling while monosaccharides react with greater velocity. The test is therefore used as a rough distinction between mono- and disaccharides. Reduction is shown by the precipitation of red cuprous oxide as in Fehling's test.

To the reduction tests we owe our knowledge of the free aldehyde and ketone groups of the sugars. Practically, they are very useful in the detection and quantitative estimation of sugars. Their applications to urine and food analysis will be found described in laboratory manuals of biochemistry. All the monosaccharides and all disaccharides, except saccharose, respond to reduction tests. Saccharose fails to give it because its constituent monosaccharides, dextrose and levulose, are so joined together as to leave neither aldehyde nor ketone group free to react, that is, to be oxidized. None of the more complex carbohydrates give reduction tests, because condensation of monosaccharides masks the free aldehyde and ketone groups and renders them less apt to be oxidized. That polysaccharides as such are ever oxidized in plants and animals has not been proved. Seemingly, they are always reconverted into the sugar before being utilized.

The diffusibility of the sugars may be readily demonstrated by use of the *dialysis* test. Parchment paper is commonly used but collodion and other preparations may serve as a semipermeable membrane. The polysaccharides are non-diffusible, owing to their colloidal character. (See pp. 109-111.)

The sugars are not precipitated by alcohol; but since the polysaccharides are, *alcohol precipitation* serves as a general method for the separation of polysaccharides from sugars.

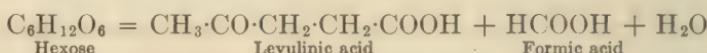
Molisch's test is given by all carbohydrates. The solution to be tested is treated with a few drops of a solution of α -naphthol and is then stratified above concentrated H_2SO_4 in a test tube. A red ring results at the zone of contact. The reaction is due to the liberation of furfural,



¹ Barfoed's solution is prepared as follows: 66.5 g. of crystallized neutral copper acetate are dissolved in 1,000 cc. water, filtered if necessary and to each 200 cc. of filtrate 5 cc. of 38 per cent acetic acid are added.

carbohydrates. It is furfural, reacting with α -naphthol, which gives the red coloration. If the carbohydrate is a pentose or a polysaccharide that yields pentose on hydrolysis (pentosan or similar complex), it will yield furfural on boiling with 12 per cent HCl or other dilute mineral acid. As furfural is volatile, it may be distilled off during the boiling and detected qualitatively by its power to redden aniline acetate paper or determined quantitatively by precipitation as a furfural-phloroglucin compound, which may be filtered and weighed. The quantities of pentose and pentosan in animal and plant tissues have been determined in this way, since the only substance besides pentose to yield furfural during boiling with dilute mineral acid is glycuronic acid and this is not commonly present in pentose-yielding structures. The *furfural distillation test*, then, is a general one for pentoses.

An equally specific test for hexoses, is based upon the fact that during prolonged boiling with dilute HCl hexoses yield *levulinic acid* and formic acid. The reaction is given as follows:



Levulinic acid may be recognized by the preparation of its crystalline silver salt after treatment with AgNO_3 .

A reaction which serves to distinguish the ketoses from the aldoses is *Seliwanoff's test*. The solution to be tested is treated with an equal volume of a reagent prepared by adding a small amount of concentrated HCl to a dilute solution of resorcinol in glacial acetic acid. On heating, a red color is produced in the presence of ketoses.

The so-called *caramel test* is obtained by heating dry sugars or very concentrated sugar solutions. This produces a brown color and the characteristic caramel odor. The resulting mass is a complex mixture of various oxidation products and is used as a coloring and flavoring substance for confections and other foods.

The *mucic acid test* is specific for galactose. It is performed by treating the sugar solution with an equal volume of concentrated HNO_3 and evaporating to a small volume, when a white precipitate of mucic acid results from the oxidative action of HNO_3 on galactose. Lactose (milk sugar) and certain other carbohydrates which, by hydrolysis occurring in hot acid solution, may yield galactose also give this test. Sugars other than galactose do not oxidize to give mucic acid. As it is oftentimes necessary to distinguish between dextrose and lactose in human urine, the test is of practical value to confirm the osazone reaction, and the fermentation and other tests.

The *iodine test* is very useful in the identification of certain carbohydrates. The solution used, Lugol's, is metallic iodine dissolved in dilute KI. The reaction with starch to give an intense blue color is a very deli-

cate test. The blue starch iodine is of unknown composition. It forms in neutral or acid solution but not in the presence of alkali or alcohol. It must be a loose compound, since heating it causes dissociation with disappearance of the blue color, which reappears on cooling. Presumably, iodine is held only upon the surfaces of the large colloidal aggregates of starch so as to give the physico-chemical combination known as an adsorption compound. Starch in its natural state (small hard grains) or in the colloidal state (starch paste or soluble starch) responds to the test. The only other substance, giving a blue iodine test is the so-called amyloid, formed by the action of sulphuric acid on cellulose. Glycogen responds to Lugol's solution with a deep brownish red color, usually described as a port-wine red. This reaction is also prevented by the presence of alkali or alcohol and by heat. It requires the presence of inorganic salts and a comparatively high concentration of iodine. Certain of the dextrines also give a red color in this test, but not the same shade seen in the glycogen reaction. The dextrins, that so react, are accordingly known as erythro-dextrins (from the Greek for red).

Fermentation of the carbohydrates may be caused by various micro-organisms. Yeast fermentation, producing alcohol and carbon dioxide as the chief products, with lactic, butyric, and other organic acids in traces, is useful in differentiating lactose (milk sugar) from the other common sugars. Ordinary bread yeast or brewers' yeast ferments all the common sugars except lactose. As yeast fermentation appears to be a change of monosaccharides to alcohol and carbon dioxide, the fact that saccharose and maltose are readily fermented indicates that these disaccharides are split by the yeast before being fermented. Many other types of yeast are also effective in fermenting the sugars. An Asiatic fungus, used in the form of kephir grains, produces an alcoholic fermentation of lactose quite similar to the action of beer yeast on maltose. The alcoholic carbonated drink, kouymiss, is prepared by kephir fermentation of mare's milk. This fungus can hydrolyze lactose though ordinary yeasts cannot do so. Yeast fermentation is a practical qualitative test to differentiate between dextrose and lactose in the diagnostic analysis of human urine.

Bacteria, like yeasts, vary in their ability to ferment various carbohydrates; but apparently, every carbohydrate is fermented by at least some types of bacteria. The differing fermentative powers of different bacterial species reveal, as do also those of different yeasts, the specific asymmetry of living matter. Such different powers are due to the fact that bacteria and yeasts possess enzymes which are highly specific in their ability to ferment different stereoisomeric modifications of sugars. Because of this fact, different strains of bacteria are distinguished from each other, in laboratory practice, by tests that show what particular carbohydrates can be fermented by each strain.

The bacterial fermentation of polysaccharides in animal intestines changes such insoluble substances as cellulose and hemicellulose, that are unaffected by animal digestive juices, into the soluble monosaccharides, which may be absorbed and utilized by the animal. The sugars, so formed, as well as any other sugar present in the intestine, may be subjected to further bacterial fermentation, so as to produce small amounts of alcohol and carbon dioxide. Sugars are much more often subjected to *acid fermentation* in the intestine. During this process, fatty acids, lactic acid and other organic acids are produced. When the contents of the lower intestine are acid, it is almost invariably due to this cause. The formation of lactic acid from milk sugar is a prominent factor in the process and is effected by numerous kinds of microorganisms, many of them ordinarily present in milk. The lactic acid bacillus, *B-Acidi-lactici*, is especially abundant.

Gases, in addition to CO_2 , are also formed by fermentation of the carbohydrates. Methane or marsh gas (CH_4) and hydrogen are the most important.

Bacterial inhabitants of the intestines are not without value to the host. They enable celluloses to be utilized. In herbivorous animals, provided as they are with a large cecum and long intestines, capable of permitting prolonged fermentation, foods rich in cellulose are rendered of nutritive value and constitute one of the chief foods of such animals. In human beings, only a little cellulose from the softer portions of plant structures is probably utilized.

Hydrolysis of the Polysaccharides.—The various polysaccharides are, in large measure, identified and described according to the products of hydrolysis. A general method of procedure is to boil for a number of hours with dilute mineral acid. Combinations of the sugars other than polysaccharides, combinations such as glucosides, sugar-containing proteins, etc., may be similarly treated. After complete hydrolysis, the resulting monosaccharide may be identified by the osazone test or by rotatory power or by specific chemical tests in some cases. Pentoses may be distinguished from hexoses by furfural distillation from the former and by levulinic acid production from the latter. The progress of hydrolysis can be followed, in some cases, so as to show that the process occurs in a series of reactions. The hydrolysis of starch, for example, proceeds in such a way that the following successive stages are detected:

1. Starch—gives a cloudy solution, "starch paste."
2. Soluble starch—clear solution, still gives blue color with iodine.
3. Amylo-dextrin—purple color with iodine, is probably a mixture of soluble starch and erythro-dextrin.
4. Erythro-dextrin—red color with iodine.
5. α -achroodextrin—no color with iodine, precipitated by alcohol.

6. β -achroodextrin—no color with iodine, precipitated by higher concentrations of alcohol than are required for α -achroodextrin.
7. Other dextrans of ill-defined character, require still higher concentrations of alcohol for precipitation and show some tendency to diffuse through parchment dialyzer. Stages 1-6 are non-diffusible.
8. Maltose—a disaccharide.
9. d-Dextrose—a monosaccharide.

This representation of the process shows that the large starch molecule, or aggregate of molecules, is gradually broken up, so that substances of progressively smaller molecular weight and greater diffusibility appear. There is some evidence that a part of the process, at least, consists in the successive spitting off of maltose, one molecule at a time, since maltose may be detected early in the hydrolysis, when erythro-dextrin is still present. Maltose production cannot be detected during starch hydrolysis by acids, because the maltose, as soon as it is produced, is further hydrolyzed into dextrose. But during hydrolysis by certain enzymes, such as diastase of malted grains, maltose is the only sugar produced; so that with the enzymes, the progress of hydrolysis can be followed satisfactorily and the early production of maltose can be detected.

Starch hydrolysis illustrates the general tendency of life processes to take place in a series of linked reactions. The hydrolysis of many other polysaccharides and also of proteins is analogous to that of starch. Proteins pass through metaprotein, proteose, peptone and peptide stages before the entire material is hydrolyzed to amino-acids.

The Colloidal Character of the Polysaccharides.—Most of the polysaccharides, as found in nature, exist in the colloidal state. Starch is stored in plant cells in the form of hard distinct grains of microscopic size. Close inspection of individual grains shows them to be composed of concentric layers, so that striations can be plainly made out. Apparently a nucleus, seen as a tiny dot within the grain, serves as the starting point for starch deposition which then proceeds layer by layer. A very small amount of cellulose is found even in the most carefully purified starch grains.

Separation of starch from seeds, tubers, etc. is a simple process. The ground, pulped material is mixed with a large volume of water and allowed to stand either quietly or with very slight agitation. The starch grains, because of their high specific gravity, rapidly settle and may be collected and further purified by repeated washing with cold water.

The appearance of the grains is distinctive for every kind of plant. Variations in their shape, size and markings and in their appearance in a microscope field, illuminated by polarized light, enable one to identify the natural source of any preparation of starch. Identification of starch

by microscopic inspection is utilized in the detection of many kinds of food adulteration. The appearance of the grains of some typical starches is shown in Fig. 26.

When starch grains are subjected to moist heat and particularly when surrounded by boiling water, they are disrupted so that the con-



FIG. 26.—Microscopic appearance of starch grains. 1, Potato. 2, bean. 3, arrowroot. 4, rye. 5, barley. 6, oat. 7, rice. 8, maize. 9, wheat. (After Leffmann and Beam).

tents pass into a colloidal state. The result, known as starch paste, is not a highly dispersed colloid; but contains the starch molecules in a state of aggregation—large particles of emulsoid colloid. For this reason, starch paste settles on long standing, so that a highly concentrated paste is at the bottom and clear water, containing no more than a trace of starch, forms the upper layer. On prolonged boiling, however,

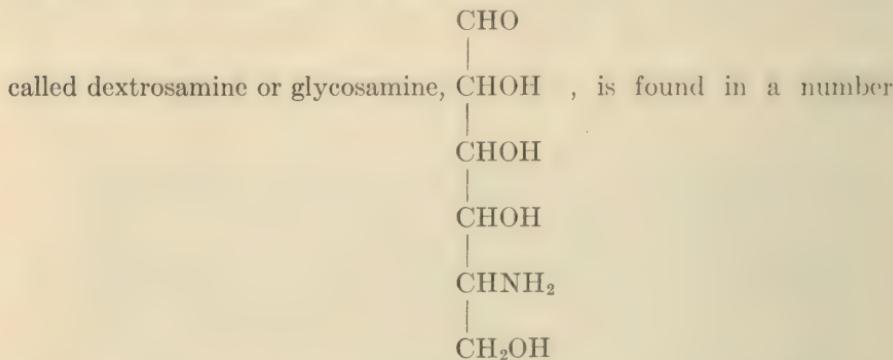
particularly in the presence of a very little acid, starch paste is transformed into soluble starch and in this condition, gives a true colloidal solution of more highly dispersed character. Though it still gives the typical test with iodine, its solutions are nearly or quite transparent; but the material is non-diffusible and shows a bright cloud in the field of the ultramicroscope. The further hydrolysis of soluble starch produces a series of dextrans of progressively less pronounced colloidal character. The later stages of the process produce small molecules which are in true solution and diffuse through parchment paper.

Glycogen is also a typically colloidal polysaccharide. Glycogen is the common storage carbohydrate in animals as starch is in plants. Its solutions are of a milky opalescence with a marked Tyndall effect and are non-diffusible. The stages in the hydrolysis of glycogen to dextrose have not been worked out, but intermediate substances are known to exist.

A number of polysaccharides show their colloidal character by *gel formation*. *Pectin*, obtained by partial hydrolysis of fruit pectoses in boiling water, gives the gelling properties to fruit jellies. The pectoses are complex substances, possibly mixtures of polysaccharides, found in numerous varieties of fruits and some other plant structures. The calcium salt of pectic acid which is present in pectin at the right stage of hydrolysis is necessary for gel formation. *Lichenin*, the chief carbohydrate of Iceland moss, is used in the preparation of blanc mange because of its gel production. Similarly, *agar-agar* obtained from several varieties of seaweeds, is employed in bacteriological and other culture media to give the gel consistency. Both lichenin and agar-agar are not digested by human beings because the enzymes of the digestive system do not affect them. This is particularly surprising in the case of lichenin, since by acid hydrolysis it is shown to be capable of yielding only dextrose. It is an anhydride of dextrose in the same sense that starch is, but apparently lichenin and starch are differently constituted. The fact that lichins have nutritive value for the reindeer deserves investigation of the digestive powers of this species. Agar-agar yields chiefly galactose on hydrolysis; but arabinose is also obtained, so that agar-agar is difficult to classify either as a hexosan or a pentosan. It may be a mixture. Closely associated with it in the various marine algae that yield it, is the substance *algin*, a carbohydrate-like complex, which behaves as a weak acid and is therefore sometimes called *alginic acid*. It probably has much the same relation to carbohydrate gel formation that pectic acid has, since its calcium salt forms a very stiff gel. The residue, obtained by drying this gel is so highly insoluble as to be of great practical use in waterproofing.

Some Important Derivatives of Sugars.—Several of the sugars, especially dextrose, are found in the form of important derivatives not readily

recognized as carbohydrate in character. Dextrose, in an amino form,



of proteins. An example of these proteins is *mucin*, which gives the sticky, mucilaginous consistency to saliva and to mucus secretions. Mucin may be precipitated by acidifying saliva with acetic acid. After it is thus separated, it hydrolyzes in boiling dilute HCl to yield amino-acids and dextrosamine. The latter reduces Fehling's solution and has many characteristics of dextrose, but also gives reactions for the amino group. Because of this group it can combine with acids. With HCl, for example, it forms dextrosamine hydrochloride. By means of its reactions, dextrosamine is shown to have the structure represented above. Proteins, similar to mucin and called the *mucoids*, are found in tendon, cartilage and bone. They hydrolyze to yield galactosamine which is analogous to dextrosamine. *Chitin*, the hard substance of lobster and crab shells, indeed of the exoskeleton of all arthropods, is a complex anhydride of dextrosamine, so that the latter is found in large amount among the hydrolysis products of chitin.

Another sugar complex occurs in the *nucleoproteins*, which are found in cell nuclei though also occurring in small amounts in cytoplasm. Nucleoproteins yield *nucleic acids* after mild hydrolysis. (See classification of proteins, Chap. II.) Nucleic acids are complex substances that break down during acid hydrolysis to yield: Phosphoric acid, four different nitrogen-containing bases and a sugar. The latter is either d-ribose (aldo-pentose) or a hexose not yet identified. Ribose is obtained from plant nucleic acids, prepared from yeast and wheat, the hexose is obtained from animal nucleic acids, prepared from thymus gland, pancreas, etc.

The *cerebrosides*, mentioned in Chap. III, as abundant constituents of nervous tissue are sugar-containing compounds, since they hydrolyze to yield galactose.

The *glucosides* of plants are also sugar complexes and constitute a large and heterogeneous group. They are compounds of glucose with various different things, in most cases a nitrogen-containing base. They include the active principles of many essential oils; examples: *Sinigrin*

(from black mustard oil) and *sinalbin* (from white mustard oil) of mustard seed; also the highly flavored substance with a burning taste in horseradish. *Amygdalin* in oil of bitter almonds and in cherry and peach stones is also a glucoside. Amygdalin contains the hydrocyanic acid radicle and has an odor somewhat resembling potassium cyanide. Many important drugs of plant origin are glucosides: *Saponin* from various plants (soapbark, soapwort, cyclamin, nightshade and sarsaparilla) has a remarkable power to lower surface tension and is a powerful hemolysin; *salacin*, from the willow-tree, is an antipyretic; *phloridzin*, from the roots of cherry and certain other fruit trees, so affects the kidney as to cause glycosuria; *digitoxin*, from the foxglove, is an active principle of digitonin, used as a powerful heart stimulant. A number of others have been discovered. There seems to be a general tendency in plant structures to produce complex syntheses of dextrose with substances, both basic and acidic in character.

This list of sugar derivatives is by no means exhaustive. It merely includes those which have received special study. It is sufficient, however, to illustrate the extensive rôle which carbohydrate plays in vital chemistry. The list shows that the carbohydrate group appears as a constituent of proteins and of lipoids, as esters (that is, combined with acids) and as the more typical glucosides which have it in combination with bases. To a very mild degree, then, a sugar molecule is amphoteric.

Carbohydrates as Fuel and as Storage Material.—The most striking value of carbohydrate in the economy of living things is its utility in oxidation processes. In both plants and animals, sugar oxidizes more rapidly and completely than do other substances. Although animal foods include a diversity of polysaccharides and sugars, they are all transformed into monosaccharides during digestion before they are absorbed from the intestine into the blood. Since the bulk of carbohydrate food consists of dextrans, dextrose is the chief monosaccharide produced in digestion. The other monosaccharides, however, are transformed into dextrose in the liver and possibly in other organs; so that dextrose is the only sugar normally present, as such, in the body. Its oxidation produces a number of intermediary substances but the final products are $\text{CO}_2 + \text{H}_2\text{O}$. It is the chief and, under most circumstances, the only substance oxidized to furnish energy for muscular work. The stimulating, or at least restorative, effect of carbohydrate food is very marked in a fatigued human being. This is especially true of sugars, since they are quickly absorbed. The craving for sugar, as felt by a person taking vigorous exercise or by a rapidly growing child, is a normal and physiological phenomenon. There is some evidence to indicate that a mammal cannot be long maintained in good physiological condition on a carbohydrate-free diet. Conclusive evidence is difficult to obtain, because of the ability of the animal body to transform fat and, to a lesser degree, protein into

DESCRIPTIVE DATA CONCERNING SOME OF THE MORE IMPORTANT NATURAL CARBOHYDRATES

Names	Type	Formula	Occurrence in nature	Osazone	(α) ^b of 10 per cent solution at 20°C.	Reduction tests	Distinctive tests	Diffusibility through parchment	Products of hydrolysis
1 d-Glucose, dextrose, grape sugar	aldo-hexose	C ₆ H ₁₂ O ₆	All animal tissues and juices, plant juices and combined in many forms.	dextrosozone	+ 52.5	+	+	dextrose
2 d-Fructose, levulose, fruit sugar	keto-hexose	C ₆ H ₁₂ O ₆	Many plant juices and honey; combined in sucrose and inulin.	dextrosozone	- 92.3	+	Seliwanoff test	+	fructose
3 d-Mannose	aldo-hexose	C ₆ H ₁₂ O ₆	Combined in a few plant polysaccharides.	dextrosozone	+ 14.2	+	+	mannose
4 d-Galactose	aldo-hexose	C ₆ H ₁₂ O ₆	Combined in lactose, certain vegetable gums and cerebrosides of brain.	galactosazone	+ 80.5	+	Mucic acid test	+	galactose
5 L-Arabinose	aldo-pentose	C ₅ H ₁₀ O ₆	Combined in gum arabic and cherry gum.	1-arabinosazone	+ 104.5	+	+	arabinose
6 L-Xylose, wood sugar	aldo-pentose	C ₅ H ₁₀ O ₆	Combined in wood gums.	l-xylosazone	+ 19.0	+	+	xylose
7 d-Ribose	aldo-pentose	C ₅ H ₁₀ O ₆	Combined in plant nucleic acids and their derivatives.	d-arabiosazone	- 18.8	+	+	ribose
8 Saccharose, sucrose, cane sugar hexoses	disaccharide, 2 hexoses	C ₁₂ H ₂₂ O ₁₁	Sweet juices of plants.	dextrosozone after hydrolysis	+ 66.5	-	Sweet taste	+	glucose fructose
9 Maltose, malt sugar, grain sugar	disaccharide, 2 hexoses	C ₁₂ H ₂₂ O ₁₁	Malted grains and other products of partial hydrolysis of starch.	maltoazone	+ 140.0	+	+	glucose

10 Lactose, milk sugar	disaccharide hexoses	$C_{12}H_{22}O_{11}$	Milk	lactose zone	+ 52.5	+	Mucic acid test	+	glucose galactose
11 Raffinose, melli- tose	trisaccharide hexoses	$C_6H_{12}O_{16}$	Australian manna, cotton seed, molasses, small amounts in many plants.	strongly +	-	+	glucose fructose galactose	
12 Glycogen	polysaccharide dextran	$(C_6H_{10}O_5)_x$	All animal tissues and yeast.	about +200	-	Red color with iodine	-	glucose
13 Starches	polysaccharide dextran	$(C_6H_{10}O_5)_x$	Storage parts of most plants.	+190.21	-	Blue color with iodine	glucose
14 Dextrines	polysaccharide dextran	$(C_6H_{10}O_8)_x$	In products of partial hydrolysis of starch.	about +195	-	Red with iodine in some cases	-	glucose
15 Celluloses	polysaccharide dextran	$(C_6H_{10}O_8)_x$	Harder parts of plants.	-	glucose
16 Hemi-celluloses	polysaccharide unknown	unknown	Harder parts of plants.	-	pentose hexose
17 Inulin	polysaccharide levulan	$(C_6H_{10}O_8)_x$	Storage parts of <i>Com-</i> <i>positae</i> .	strongly -	-	Easily hydro- lyzed	-	fructose	
18 Agar-agar	polysaccharide mixed type	unknown	Various sea weeds.	-	Gel formation	-	galactose arabinose	
19 Lichenin	polysaccharide dextran	$(C_6H_{10}O_5)_x$	Iceland moss.	-	Gel formation	-	glucose	
20 Gum arabic	polysaccharide araban partly	unknown	Certain tree gums.	-	mostly arabinose	

¹ For soluble starch at 17.5°C.

sugar. The utilization of even a little carbohydrate seems to facilitate the metabolism of all substances in the body. Its usefulness as a fuel is further enhanced by the facility with which carbohydrate may be stored. It is packed away in the form of starch in most plants or as inulin in the dahlia, sunflower, burdock, and all other members of the *Compositae* group. The chief storage tissues are roots, tubers, nuts, fruits and the endosperm of seeds. In animals, it is stored in the form of glycogen which accumulates on a carbohydrate-rich diet in various tissues, but chiefly in the liver, with muscles next in order. During periods of great muscular activity or of increased heat production to withstand exposure to cold or during periods of carbohydrate deficiency in the diet or during starvation, glycogen is rapidly transformed into dextrose and oxidized. Details of the story of carbohydrate metabolism will be given in another connection.

The table on pages 114 and 115 will serve to summarize various facts relating to the more abundant carbohydrates found in nature.

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CHAPTER V

THE INORGANIC CONSTITUENTS OF LIVING MATTER

In addition to proteins, fats, carbohydrates and other typical organic compounds, mineral substances or so-called inorganic constituents are also of prime importance to life. Many of the latter exist in living things as components of carbon compounds or, as commonly expressed, in organic combination. In this state they are not recognizable by the ordinary tests for mineral substances, but must be freed from organic combinations. To accomplish this, the material is generally burned. The carbon compounds are thus oxidized and volatilized forming chiefly CO_2 and H_2O . Nitrogen is simultaneously driven off as ammonia or as nitrogenous bases or as ammonium salts. The typical inorganic constituents, however, tend to remain as an ash at ordinary burning or gas flame temperatures. The "wet-ash" method may also be applied. In this procedure the substance to be examined is heated with an excess of concentrated mineral acid until all organic matter is completely oxidized and only inorganic constituents are left in the acid. It is from analysis of the ash of animal and plant structures that information as to the presence of inorganic material has been obtained.

The Elements Contained in Inorganic Constituents of Living Things. At least eighteen different elements (in addition to carbon, hydrogen, oxygen and nitrogen) have been recognized as constituents of organisms by ash analysis. *Sodium, potassium, calcium, magnesium, iron, phosphorus, sulphur and chlorine* are found in the tissues and fluids of all living things; *fluorine, silicon and iodine* have been recognized in many plants and animals but only in certain tissues; *copper, manganese, zinc* and *bromine* have been found in marine invertebrates and manganese and zinc are not infrequently found in plants; and lastly, certain elements, such as *barium, lithium, and arsenic*, that have been discovered in a few organisms, have not been shown to be useful for life, but are supposed to be present merely because they occur in the waters of certain localities and are stored in living tissues in harmless form, since elimination is not always rapid enough to keep the organism rid of them. This seems undoubtedly the case with arsenic, a highly toxic metal. The occurrence and significance of barium and lithium have not been so well investigated.

Inorganic Requirements of Moulds.—The inorganic substances, obtained by organisms largely in the form of mineral salts, are numerous and diverse; but the list of really indispensable ones is different for different organisms.

For *moulds*, a relatively simple assortment of salts is adequate for limited growth. Common laboratory experience shows that moulds grow in dilute solutions of many chemically pure reagents, as for example, sugar, lactic acid or phosphates. Most of the inorganic material, available for the moulds in such cases, is composed of the small amounts of sodium and potassium silicate with traces of other mineral matter dissolved from the walls of the glass container, so that the available supply must be very meager both qualitatively and quantitatively.

Raulin, about 1870, made careful experiments on a common mould, *Aspergillus*, to determine the amount of its growth in solutions varying as to the assortment and concentrations of salts present. Such experiments showed that, although a certain amount of growth may be obtained in solutions of simple composition, the moulds do not really thrive and reproduce without a more complex supply of food materials. The optimum growth was obtained in a solution of the following composition:

Water.....	1,500.00	Magnesium carbonate.....	0.40
Cane sugar.....	70.00	Ammonium sulphate.....	0.25
Tartaric acid.....	4.00	Zinc sulphate.....	0.07
Ammonium nitrate....	4.00	Ferrous sulphate.....	0.07
Ammonium phosphate.....	0.60	Potassium silicate.....	0.07
Potassium carbonate..	0.60		

If any of these constituents was omitted or used in quite different proportions, less growth was obtained. Some of these compounds are, of course, foods in the ordinary sense. The sugar is available for energy production and for various synthetic processes, ammonium nitrate and phosphate for protein and lipoid synthesis, etc.; but many of the nutrient substances appear to have more specific functions. Of the chemical elements present, those which more commonly enter into inorganic compounds are K, P, Mg, S, Zn, Fe, and Si. Three elements, Na, Ca and Cl, which generally occur in living structures, do not appear to have been in the solution. A small amount of all three was doubtless present, however, owing to the difficulties involved in the complete purification of laboratory reagents so as to obtain or keep them free from these very common elements. The use of zinc, not commonly recognized as of physiological value, is particularly notable.

Mould growth was later subjected to more detailed investigation by Bertrand and Javillier (1912). Raulin, studying the surprising effects of small amounts of zinc, had also noted the possibility that manganese might be of importance; though he was unable to establish this point since he was not sure that he used manganese absolutely free from traces of zinc; but Bertrand and Javillier, working with salts of satisfactory purity, proved that both zinc and manganese have undoubtedly importance. The effects of almost unbelievably small quantities of these elements were demonstrated. One part of zinc in 25 million parts of culture solution

caused an increase of over 50 per cent in the weight of the crop of mould growth and one part in 10 million doubled it. One part of manganese in one million parts of solution distinctly increased growth, though better effects were obtained with higher concentrations up to one part of Mn in 100 parts of culture solution. With both Zn and Mn present, still better effects were obtained. The results of one experiment will serve to illustrate:

	WEIGHT OF CROP
Control, without Zn or Mn.....	1.45
With Zn, 1:500,000	4.16
With Mn 1:5,000	2.79
With both Zn and Mn.....	4.35

The specific activity of mould growth favored by the presence of manganese was shown by Bertrand to be fructification (conidia formation) which, in spite of abundant mycelium growth, cannot occur in the complete absence of manganese. The amounts of iron required for optimum growth were also found to be extremely minute. The indispensability of certain food substances even though they are only required in infinitesimal quantities, is one of the most startling facts of physiology. We shall meet with it again, especially in connection with vitamins (Chap. VIII). One reason why such minute amounts of certain metallic elements are effective has been suggested by the observation that in some instances, iron, zinc, and manganese appear to aid in catalysis. A substance that causes catalysis enters into a reaction, but emerges from the process unchanged so that a small quantity of it may react over and over again without being destroyed.

Inorganic Requirements of Higher Plants.—For higher plants, a study of the salt requirements is obviously of prime importance in practical agriculture. Soil analysis and the correction of soil deficiency by use of fertilizers are practices which become efficient only when done in accordance with exact knowledge of the specific requirements of each kind of crop. As the subject is a large one, the reader is referred to works on agricultural chemistry for adequate treatment. Nitrates and, to a lesser degree, ammonium compounds are obviously required for synthesis of plant proteins, while phosphates, potassium, calcium, magnesium and iron in suitable forms are also indispensable. Silicon is quite generally present but does not seem to be required. Sodium and chlorine, though very abundant in sea water and always present in terrestrial waters, are not found in large amounts in plants. Sodium, at least, does not seem to be required for plant life and growth. The requirements of plants for calcium, magnesium and potassium have received especially detailed study.

Salt Requirements of Animals in General.—For animals, the requirements of inorganic salts have been surmised from a study of the ash of milk because it is adequate food for the young mammal. Von Bunge has shown, moreover, that the composition of milk ash much more closely approximated that of the body tissues than of the blood fluid. The proportions given in the following table will illustrate the point.

Ash constituent	Amounts present in 100 parts of ash		
	Entire body of young pup	Dog's milk	Dog's blood serum
K ₂ O.....	8.5	10.7	2.4
Na ₂ O.....	8.2	6.1	52.1
CaO.....	35.8	34.4	2.1
MgO.....	1.6	1.5	0.5
Fe ₂ O ₃	0.34	0.14	0.12
P ₂ O ₅	39.8	37.5	5.9
Cl.....	7.3	12.4	47.6

Compared with the ash of the body, the only marked disproportion among inorganic constituents of milk is its iron content. This is one reason, though there may be others, why milk is not satisfactory as the sole food of mammals after the early stages of growth when the reserve store of iron, present in the body at birth, has become depleted.

The blood fluid, from which the mammary gland must draw its materials, is comparatively rich in sodium and chlorine but poor in calcium, potassium and phosphate, while milk ash has all of these constituents in proportions resembling those found in body ash. The mammary gland thus furnishes a good example of the tendency, already noted, for living cells to "select" materials from environment in accord with their specific needs.

In addition to the substances tabulated, the inorganic constituents in the ash of milk comprise sulphates and exceedingly minute traces of zinc, silicates, fluorides and iodine. Milk ash has been imitated by artificial mixtures that were adequate for all the inorganic requirements of rats and other laboratory animals from the time of weaning until normal death from old age, including periods of normal reproduction. Several successful salt mixtures have been devised and found adequate when fed in a diet whose organic components were purified proteins, fats and carbohydrates together with minute amounts of vitamin-containing preparations. The following is a salt mixture devised by Osborne and Mendel:

	GRAMS		GRAMS
CaCO ₃	134.8	Citric acid: H ₂ O.....	111.1
MgCO ₃	24.2	Fe citrate: 1.5H ₂ O.....	63.4
Na ₂ CO ₃	34.2	KI.....	0.020
K ₂ CO ₃	141.3	MnSO ₄	0.079
H ₃ PO ₄	103.2	NaF.....	0.248
HCl.....	53.4	K ₂ Al ₂ (SO ₄) ₂	0.245
H ₂ SO ₄	9.2		

Sodium Chloride Hunger.—Animals vary as to their demand for sodium in the form of common salt. Adult human beings consume on the average about 10 grams per day, but this amount is much larger than the requirements of health and freedom from "salt hunger" demand. It has been shown that, with any ordinary diet, approximately 2 grams per day are sufficient. Vegetarians unquestionably require more salt than persons on a predominately meat diet. Von Bunge has made an extended study of the reported habits of the different races of men with regard to salt consumption and has shown that those, whose diet is predominately vegetarian, demand salt. Among West African negro tribes, living chiefly on vegetables, the demand for salt is insistent. As salt is scarce in those regions, it is considered one of the most valuable of all possessions and the greatest of delicacies. Children are even seen sucking a piece of salt with the relish shown by most children for sugar. The explorer, Park, reports of his travels in this region,

I myself have found the scarcity of this natural product (salt) very trying. Constant vegetable food causes a painful longing for salt that is quite indescribable. On the coast of Sierre Leone the desire for salt is so keen among the negroes that they gave away wives, children and everything else that was dear to them, in return for it.

On the other hand there are various nomadic tribes of hunters in various parts of the world, and other peoples who live on an almost exclusively meat diet, who do not seem to care for salt. In fact, travellers have found whole groups who had never tasted salt and when induced to try it found it very disagreeable. Stephanson, the explorer, reports that, while living among the carnivorous Eskimos, he was somewhat embarrassed by visits from the natives who, in accord with their hospitable customs, expected to be fed. He found that he could avoid the threatened depletion of his stores by salting the food offered to his guests. Similar contrasts are seen among animals both wild and domestic. Many herbivores, deer, wild cattle, etc., will travel surprising distances to reach natural salt deposits known as "salt licks"; while carnivora, lions, tigers, etc., have never been known to take salt. In agricultural practice, salt is supplied to cattle and horses regularly, though dogs and other domesticated carnivora do not require it.

Von Bunge has explained "salt hunger," accompanying a vegetarian diet, on the basis of the high potassium content of plant structures. The

selective storage of potassium with little or no sodium causes plants to be very rich in the former. This is particularly notable in the potato and, correspondingly, potatoes are particularly unpalatable without salt. Those communities subsisting very largely on potatoes are shown, statistically, to be large consumers of salt. The preponderance of potassium in vegetable foods tends to increase the amount of potassium in the blood beyond its customary low concentration. This reacts with sodium salts of the blood, chiefly chloride, to replace sodium by potassium and causes increased elimination of sodium compounds in the urine. This has been proved by examination of the urine after eating known amounts of potassium salts. Quantities, corresponding to the potassium found in one day's average allowance of vegetables in a vegetarian diet, cause a marked increase in the excretion of sodium in the day's urine. In short, any abnormal excess of potassium robs the body of sodium which is eliminated chiefly in the form of NaCl. On a meat diet the disproportion between potassium and sodium is not so great as in vegetable foods and does not create a "salt hunger." Not all herbivorous animals, however, crave salt. Rabbits, for example, do not seem to require it and all domesticated herbivora appear to thrive very well if not given any salt, although the eagerness with which they take it, when offered, is unmistakeable. Such animals do not consume as large an excess of potassium as do the human beings whose diet consists largely of potatoes, yet all herbivora undoubtedly consume a certain excess of potassium over sodium. These facts indicate that the physiological mechanism of hunger for sodium chloride cannot be regarded as entirely explained.

One reason why sodium chloride is required in animal food is shown by the following facts. Blood plasma always contains about 0.5 per cent of sodium chloride and maintains this proportion constant, except for temporary fluctuations within very narrow limits. Any changes in the amount of salt ingested are immediately followed by corresponding adjustments of the amount excreted in the urine. Now sodium chloride, more than any other one constituent, tends to determine the osmotic pressure of blood. Comparative constancy of osmotic pressure of blood must be maintained in the body of a mammal at all costs. Some excretion of sodium chloride, even though it be reduced to mere traces, will continue to occur in urine and sweat on a salt-free diet; so that the body cannot indefinitely maintain its salt content in the blood without some renewals. This is particularly true, as explained above, on a diet rich in potassium salts. One fundamental reason for sodium chloride hunger, then, is clearly manifest.

The ability of the animal organism to maintain its constancy of composition in spite of variations in food supply is illustrated by the following experiment: One group of young rabbits is fed during $3\frac{1}{2}$ months exclusively on hay which has a very high proportion of potassium to sodium

while a similar group of rabbits is meantime maintained exclusively on cow's milk which has sodium in higher proportion than potassium. At the end of the experiment, blood and tissues are collected from both groups and analyzed with the surprising result that both give identical analyses for sodium and potassium. Not only blood, then, but body tissues as well are regulated to maintain constant proportions of sodium and potassium and the "selective" mechanism is capable of meeting a considerable strain in the form of great variation in the sodium and potassium supply in the food.

We ordinarily ease the strain by correcting the disproportion through addition of sodium. An interesting case of this is mentioned by Von Bunge who reports the custom of a native negro tribe near Khartoum. They burn to an ash a plant belonging to the so-called "salt wort" family and use the ashes as salt for their food. Plants of this group are among the few known to have higher sodium than potassium content in the ash. As its palatability and power to satisfy "salt hunger" are apparent, the selection of this particular plant ash as a condiment was probably not accidental.

The Animal Requirement for Iron.—This need has received considerable investigation because of its connection with the condition of *anemia*, a symptom of certain types of human malnutrition and of other pathological conditions. Iron is an essential constituent of the red protein, hemoglobin, of blood and, although iron composes only 0.0335 per cent of hemoglobin, the large quantity of it in blood results in the presence of at least 2.5 grams of iron constantly circulating in the body. In addition, iron occurs as a constant component of various body tissues: About 0.02 per cent of liver, about 0.01 per cent of heart muscle, and smaller quantities in other muscle. Indeed, minute quantities of iron regularly accompany the nucleoproteins in all tissues. The hemoglobin of the body is constantly suffering some destruction. This is indicated by the characteristic pigments of the bile, which are related to certain decomposition products of hemoglobin. From the amounts of pigment found in bile, it has been possible to estimate the quantity of hemoglobin disintegrated. In human adults, 10 per cent of the hemoglobin of the entire blood is estimated to be destroyed daily. Hemoglobin is the "respiratory pigment," which means that it is required for the transport of oxygen from lungs to the active tissues of the body. A constant new supply of hemoglobin must, therefore, be regenerated and indeed, the hemoglobin content of the blood, under normal circumstances, fluctuates only within rather narrow limits. A supply of iron for its production is constantly required. The body largely conserves the iron liberated in the liver, where hemoglobin is disintegrated and bile formed; so that the same iron is, to a considerable extent, used over and over in the economy of the animal body. This conservation is not, however, perfect. Some

iron is continually being lost, mostly with the feces and to a smaller extent with the urine; and so, for hemoglobin production, irrespective of other less definitely recognized needs, the body must obtain renewed supplies of iron. A deficiency of iron in the diet cannot long be endured without disastrous results.

Fortunately the iron requirements of living things, in general, result in the presence of useful stores of this element in foods of both plant and animal origin, especially those used in a comparatively natural, not especially refined, state. The following table shows the iron content of some foods commonly used in human dietaries:

IRON-CONTENT OF FOODS GIVEN IN PER CENT OF EDIBLE PORTION

Data from Sherman, "Chemistry of Food and Nutrition"

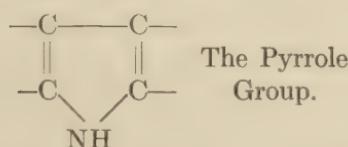
Food	Iron (Fe)	Food	Iron (Fe)
Egg-white.....	0.0001	Potatoes.....	0.0013
Butter.....	0.0002	Cheese.....	0.0013
Whole milk.....	0.00024	Dates.....	0.0030
Apples.....	0.0003	Eggs.....	0.0030
Carrots.....	0.0006	Meat.....	0.0023-0.0033
Lettuce.....	0.0007	Spinach.....	0.0036
Cornmeal.....	0.0009	Oatmeal.....	0.0038
White bread.....	0.0009	Barley.....	0.0041
Asparagus.....	0.0010	Egg-yolk.....	0.0086
Cabbage.....	0.0011	Blood.....	0.0526
Fish.....	0.0008-0.0013		

Among the most frequently used foods, eggs, meat, spinach and oatmeal are comparatively rich in iron. Further discussion of the iron requirements of human beings will be taken up in connection with dietetics.

Much investigation has been undertaken in an attempt to decide whether or not salts of iron, such as the chloride or the citrate, may serve as a satisfactory supplement to the food supply of iron. Although histological examination of the intestines, liver and spleen of animals, fed with iron salts, has shown clearly that such iron is absorbed, yet treatment of anemia by iron administration has not always met with unquestioned success. Such failures indicate that ability of the body to utilize iron supplies in the formation of new hemoglobin may be lacking; so that mere increase of available iron is not a curative measure. It has been strongly maintained that the necessary iron should be given in the form of protein combinations. Such are found in meat and egg yolk. Since the egg must contain adequate nourishment for the entire development

of the chick, an iron supply in the egg is to be expected. The iron-containing protein of egg has been regarded as an ideal source of iron for the animal body. Further investigation, however, has demonstrated that, during the process of digestion, such iron is liberated from the protein and is really absorbed in the form of inorganic iron salts giving the same tests for iron in tissues of the intestinal wall that are obtained after feeding iron salts. Beneficial results, in cases of *chlorosis*, a form of anemia common in girls at the time of puberty, and in other anemias, have been obtained by using any one of several iron salts or of organic iron compounds; so that one feels inclined to the view that, provided other physiological conditions are right for hemoglobin synthesis, iron in any form may be utilized in the process. Long continued use of inorganic iron salts is apt, at least in some individuals, to cause soreness in the intestinal lining; so that, from a practical point of view, the organic forms of iron are generally preferred as a tonic.

Our knowledge is not yet complete, however, as to just what the body needs in addition to iron in order to maintain a normal rate of blood production. One type of experiment suggests a very interesting possibility. Suppose a litter of rabbits to be divided at weaning time into three groups. One group is maintained on a milk diet which, as explained above, is very deficient in iron; a second is given milk plus a suitable allowance of inorganic iron; the third is fed on a normal diet of herbage and fresh vegetables. The first group will be found stunted in growth and defective in hemoglobin content of blood; the second, though not so backward in growth, will still be defective in hemoglobin; the third group, fed on green vegetation, show characteristically adequate blood production. Something essential is evidently supplied by the vegetable foods. Since hemoglobin, after brief heating with acids, yields a decomposition product, called hematin, which contains all the iron of the original hemoglobin, we know that its iron is built into the peculiar structure of the hematin group. Hematin will be described in more detail in connection with blood chemistry, but suffice it for the present to call attention to the fact that it contains four of the atomic arrangements known as pyrrole groups. The pyrrole group is represented thus:



There is much evidence to show that animal bodies cannot synthesize this particular grouping, but must obtain it preformed from food substances. The proteins constitute one source since proline and oxyproline, two of their constituent amino-acids, are built on the pyrrole plan; but no

animal proteins are conspicuously rich in the prolines. The chief proteins of grains, however, have an exceptionally high content of proline. Because of this, such proteins are called prolamines. Cereals, then, are a useful source of the pyrrole group. Another source is found in chlorophyll and other plant pigments since they are built on a plan similar to hematin and are rich in pyrrole groups. Thus vegetable foods, especially grain and green herbiage, should serve as sources of pyrrole groups which probably are just as necessary to the process of hemoglobin production in the animal body as is iron itself.

In the rabbit experiment just described, milk supplemented with organic salts of iron, stimulates growth even when hemoglobin production is still inadequate. Remembering that iron is widely distributed in the tissues, chiefly in the form of nucleoprotein combinations, it seems probable that iron may aid in tissue growth quite aside from its usefulness in blood production.

To summarize, then, iron, though constituting only a small proportion of the material of animal bodies, is an absolutely necessary constituent, utilized in several ways. The major part of it goes into the hematin group of the blood pigment, hemoglobin. Although the body shows considerable ability to conserve its iron supply there is some continuous loss which must be made good by feeding. Satisfactory hemoglobin production, however, cannot be assured by merely maintaining an adequate iron supply. Other needs requiring further investigation must also be met; a satisfactory supply of substances yielding the pyrrole group is probably one of these needs.

The Requirements of Animals for Calcium.—These are likewise imperative. Calcium phosphate and carbonate comprise about 95 per cent of bone ash, which amounts to about 40 per cent of the weight of dried bones; so that the amount of calcium in the bony framework alone, exclusive of the tissues and the fluids of the body, is very considerable. The needs of the young animal during active bone growth demand an especially generous supply of calcium. For mammals, milk is, of course, the chief and, during early growth, the only source of calcium. It is present in milk in two forms: As calcium phosphate and in combination with the protein casseinogen which behaves like a weak acid holding calcium in the form of a protein salt. The two forms appear, in accordance with a preponderating weight of evidence, to be equally available for animal needs. For this reason the use of lime water in diluting cow's milk for infant feeding can assure an adequate calcium supply to the child. It must be understood, however, that adequate calcium supply is not the only requisite for bone growth. Indeed, an abundance of calcium, of phosphates, and of all other inorganic materials, known to be constituents of bone, does not necessarily result in growth of properly hardened bones. The process of bone development is a complex one involving

first, the formation of a comparatively soft cartilaginous structure, resembling the future bone, and second, a process of calcification or hardening, during which inorganic material, chiefly calcium phosphate, is abundantly deposited in the cartilaginous matrix. When requirements for calcification are not fulfilled, soft bones result.

This condition is known as *rickets* and is sufficiently prevalent among ill-nourished children with bow-legs and misshapen frames to challenge physiological investigation. In extreme cases of rickets, even those bones that have been previously hardened may become soft from loss of calcium which is excreted from the body in large amounts. This may occur in spite of a high intake of food calcium. True, mere deficiency in calcium phosphate can cause a form of rickets easily cured by supplying this lack; but there are on record so many cases of this difficulty in children and in experimental laboratory animals even when the calcium supply is abundant, that knowledge of the physiological requirements for the process of bone hardening must be regarded as still incomplete. Maintenance of the proper ratio between calcium and phosphates of the food seems to be important. Curiously enough, cod liver oil is a specific cure for rickets. This fact has long been known though what constituents serve as the active agents of cure, is not yet known. Some evidence, indicating that a vitamin is concerned, will be given in Chap. VIII.

Calcium is excreted from the body in small quantities daily. Some of it is put out by the kidneys into the urine and part is secreted by the lining of the intestine and appears in the feces. Calcium excretion is increased by the ingestion of excessive quantities of magnesium in the same manner that sodium output is affected by potassium. Secretion of milk with its high content of calcium is a heavy drain on the calcium store of the maternal organism. For herbivorous animals, this loss is made good by the rich supply in green plants, and for carnivora, by eating bones; but the human mother must either depend on cow's milk or on a diet especially rich in certain vegetables. The diet of the nursing mother is frequently deficient in calcium and phosphates. During nursing periods, women not infrequently suffer from softening of the bones (*osteomalacia*) and especially from trouble with teeth (unusual tendency to decay). These difficulties are commonly attributed to a partial decalcification of bony tissues in an effort of the maternal organism to keep up the large secretion of calcium and phosphate in milk in spite of an insufficient supply of these materials in the food.

Calcium, like sodium and potassium, is always present in the blood stream in the form of dissociable salts. The proportions of these three to each other and to the total quantity of blood are maintained very constant. Fluctuations, under normal circumstances, are only within exceedingly narrow limits. The importance of this regulation can not be overemphasized. Maintenance of a proper balance between sodium,

potassium and calcium ions is an essential condition of life. One reason for this has already been considered in connection with the effects of surface tension on emulsions such as doubtless exist in protoplasm (Chap. III). The instances of the antagonistic action of calcium on sodium and potassium, as there described, have their counterpart in the behavior of these metallic ions in numerous animal processes: Maintenance of the rhythmic heart beat, of contractile power in other muscles, of irritability in nerves, etc. Description of these phenomena may be better taken up after a discussion of the behavior of the inorganic salts as electrolytes; but suffice it for the present to state that the proportion of calcium to certain other inorganic ions is regulated in all living structures.

Magnesium is also a constant constituent of animal fluids and is maintained in small but definite proportion to calcium. If this proportion is increased the tissues lose irritability and it is even possible to produce a profound and complete anaesthesia by injection of magnesium salts. Meltzer and Åuer who have extensively investigated *magnesium anaesthesia* have made the astonishing discovery that an injection of a suitable quantity of CaCl_2 during anaesthesia from MgSO_4 , rapidly and completely abolishes the magnesium effect. Obviously some type of mutual antagonism exists between the physiological effects of calcium and those of magnesium. One peculiar effect of magnesium is its power, when present in unusually high concentration in the blood, to cause some metabolic disturbance that results in glycosuria.

The Animal Requirement for Chlorine.—This is doubtless a general one, since the amount of chlorides in the blood is always large and is conserved. Any marked decrease in chlorides of the food is immediately followed by a corresponding decrease in excretion of chlorides in the urine. The effect of chlorides in maintaining osmotic pressure has already been mentioned. One specific use of chlorine is important in animal physiology: The *secretion of hydrochloric acid* in the gastric juice. Considerable quantities of chlorine are required for this purpose. Mammalian gastric juice contains some 0.2–0.4 per cent hydrochloric acid. The source must be sodium chloride of the blood which is selected for use by the peculiar acid-secreting cells in the glands of the stomach. Chlorides are also found to a greater or less extent in the ash of all tissues of the body.

Animal Requirements for Fluorine and Silicon.—These requirements are not large. Both of these elements occur, in small quantities, in bones and teeth. The significance of their presence is not known. Quite possibly silicates contribute to the rigidity and strength of bones. Silicon in the form of a complex organic silicate occurs in hair and feathers. As much as 40 per cent of the ash of hair consists of SiO_2 . Silicon is also comparatively abundant in the crystalline lens of the eye. The food sources of fluorine and silicon are: Milk which contains traces

of both, and vegetable foods whose tough or fibrous structures always contain some of these elements.

The Needs of the Animal for Phosphorus.—These requirements were indicated above. In bones and other hard tissues, calcium phosphate is a prominent constituent and the phosphate group is also essential in the nucleic acid of nucleoproteins and in lecithins and other phospholipins. In addition to phosphates of milk, phosphorus is present in the form of the phospho-protein, caseinogen, which constitutes over 3 per cent of cow's milk. Eggs contain a corresponding phospho-protein, vitellin, in the yolk. A considerable quantity of phosphates, in the form of inorganic salts, is constantly present in the tissues and body fluids, and is constantly excreted in the urine even during starvation; so that catabolic processes must cause the break down of organic phosphorus compounds, presumably nucleo-proteins, and a constant liberation of phosphates. Obviously this loss must be made good constantly. The chief food sources of phosphorus are milk, eggs and vegetable foods but a small amount is present in meat. A larger quantity occurs in fish and in the glandular tissues such as liver, kidney and sweetbreads.

Sulphur is required by animals mainly to form the sulphur-containing proteins. It will be recalled that one of the amino-acids, cystine, contains sulphur and in this form nearly all the sulphur ingested by animals is obtained. Sulphur, chiefly in the form of sulphates, is constantly excreted in the urine even during starvation. This fact signifies that tissue proteins, containing cystine, must be continually disintegrating. There is thus a constant demand for cystine-containing proteins in the food.

Since sulphur in cystine is in an only partially oxidized condition, its appearance in the blood and urine as the $-\text{SO}_4$ group signifies that sulphur undergoes oxidation in animal cells. Ordinarily, the sulphur of the food proteins is approximately equal to the sulphur of the sulphates in urine and feces. In short, the animal body maintains a balance of sulphur metabolism; but there are rare cases of the appearance of cystine in the urine of human beings showing that in such individuals the body has partially lost the power to oxidize sulphur.

Sulphates, though a waste product, are not without some usefulness to the body before they are excreted. The greater part of the sulphate is produced by oxidation in the liver and here it meets with diverse compounds brought to the liver from the intestine. Among them are numerous substances of a highly poisonous character produced by bacterial putrefaction of food proteins in the intestines. The chemistry of these toxic products will be considered in connection with a description of the bacterial action accompanying intestinal digestion. If such substances were to remain in the blood, autointoxication would result. The liver, however, is able to convert many of them into sulphates in which form

they appear to be non-toxic and are safely carried to the kidneys for excretion into the urine. Sulphates, are a *detoxicating agent*, useful in the liver.

Two other forms of sulphur are found in animals: (1) *Taurocholic acid* of bile and (2) substances of ill-defined character, called *sulphatides*, found among the lipoids of brain tissue and probably consisting of combinations of cerebrosides with phosphatides and the sulphate group.

The Iodine Requirement of Animals.—This is remarkable in that so little is needed, yet it is indispensable. Accumulating evidence indicates that ordinarily iodine occurs in detectable quantities only in the *thyroid gland*, which selects the minute amounts obtained from food and stores it in the form of a substance of unique chemical and physiological properties. It will be described in connection with the physiology of the thyroid. It has been named *thyroxine* and is required for growth and development and, possibly in some animals, even for life itself.

Iodine is obtained from the minute quantities occurring in numerous foods of plant origin, also from the traces present in milk and, to a considerable extent, from the traces in drinking water; but thyroxine itself appears to be a peculiar product of the thyroid gland alone. Curiously enough, a large store of iodine is found in sea weeds many species of which contain sufficient iodine to make their ashes a commercial source of iodine. This is surprising since the quantity of iodine in sea water cannot be considered as more than a trace. Incidentally, such sea weeds as are used for human food, Irish moss, etc., contain the richest supply of iodine of any of our foods.

Copper, though frequently found in liver and nervous tissue, has not been shown to be required by any vertebrates; but, as was mentioned in connection with the proteins (p. 17), it takes the place of iron as a constituent of the pigment, hemocyanin, of the blood of certain marine invertebrates. Hemocyanin has been chiefly studied as prepared from blood of the horse-shoe crab, *Limulus polyphemus*. Marine forms obtain copper from sea water, which always contains at least traces of copper salts. In the form of some protein combination, copper has also been found in the bodies of shellfish, oysters, clams, etc., where it is stored in a seemingly non-toxic form in the blood cells. The amount of copper in oysters, taken from waters near copper ores or in the vicinity of cities whose trade wastes contain copper, is sometimes enough to cause marked bluish-green coloration of oyster meats. This has been a practical difficulty sufficient to destroy the saleability of oysters from certain cultivated beds. The copper coloration only slowly disappears from oysters, transplanted to waters not contaminated by copper.

For plants, copper is an unusually violent protoplasmic poison. Fresh water plants, especially the algae, are very sensitive to it. The amount of copper, dissolved in water after a few copper coins had been in

it for four days, was found by Nägeli to be sufficient to kill *Spirogyra* in one minute, though it only amounted to one part of copper in 77 million parts of water. Mercury, lead, silver and some other metals are similarly toxic though not in such extreme dilutions as copper.

Because of the minute quantities effective, Nägeli gave the name "*oligodynamic action*" to the toxic effects of such metals. Various animal forms, worms, tadpoles and other living material, used in biological experiments, have been found to be almost as sensitive as plants to the toxicity of traces of heavy metals. Copper is very useful as a practical means of preventing fouling in reservoirs of water. The copper may be subsequently removed, even though present in merest traces, by suitable filtration of the water or by precipitation with calcium salts. Copper, though toxic to mammals, may be endured in small quantities since it, like other heavy metals, may be stored in minute quantities in detoxicated form in the liver. An explanation of the extreme toxicity of the heavy metals has been suggested in their precipitating action on proteins. Inasmuch as protoplasm has a fairly definite emulsion-like structure, whose integrity depends largely on the presence of proteins, anything, which removes proteins from the emulsion complex, will destroy protoplasm. Microscopic examination of either animal or plant cells, undergoing destruction by traces of copper, supports this explanation. The cells exhibit what is sometimes described as corrosion. The cytoplasm forms dense granules, supposedly of copper proteinate precipitate, and shortly the entire protoplasmic structure breaks up with eventually complete disintegration.

Although the usefulness of **zinc** and **manganese** for certain plants has been proved, as described above (p. 119), their wide-spread occurrence in the animal world has not received a physiological interpretation. The lack of these metals in certain synthetic mixtures of salts, that are used successfully in animal nutrition experiments, indicates that for mammals, at least, they are not indispensable.

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CHAPTER VI

THE ACTION OF ELECTROLYTES IN LIVING MATTER

Electrolytes and Non-electrolytes.—Inorganic constituents of living things exert many of their important physiological effects by virtue of their behavior as electrolytes. The typical electrolytes are the acids, bases and salts. They are called electrolytes because their solutions conduct the electric current. Their water solutions show especially good conductivity, although pure water itself is a very poor conductor. They confer conductivity upon their solutions because they dissociate, when dissolved, to form *ions* which by moving through the solution, carry the current. Ions are electrically charged and the process by which they are formed is therefore called electrolytic dissociation. Ions are named according to the charge they bear. Cations are positively charged and move toward the cathode under the influence of an electro-motive force; anions are negatively charged and correspondingly move toward the anode. The charges upon ions render them particularly reactive chemically. Most, perhaps all, reactions between inorganic compounds are really interactions between their constituent ions. Among organic compounds, electrolytic dissociation is not always detectable, so that organic reactions are not always represented as occurring between ions. But ions, formed by dissociation of electrolytes, are able to profoundly influence reactions that involve supposedly non-dissociated substances. Partly for this reason and partly because of physiological reactions between ions themselves, electrolytes are of fundamental significance in biochemistry.

Typical non-electrolytes are alcohols, aldehydes, ketones and certain esters. Carbohydrates and neutral fats are included among them. Generally speaking, the non-electrolytes are the organic compounds other than organic acids and bases. Solutions of non-electrolytes do not markedly conduct the electric current, because such compounds do not dissociate to the extent that electrolytes do. But the distinction between the two groups is not a sharp one. Sugar, urea, and many other typical non-electrolytes have been shown, by sufficiently delicate measurements, to be capable of very slight dissociation in watery solution; while fatty acids, amino-acids, proteins and other substances constitute an intermediate group, since they show an easily measureable dissociation which is nevertheless insignificant compared to that of typical electrolytes.

It is probable that all the compounds that take part in vital reactions are either capable of electrolytic dissociation or of being profoundly influenced in their chemical behavior by ions of other compounds. This probability is in accord with the theory of the electronic structure of matter which assumes that all atoms are composed of positive and negative electrons. Electrons are unit electric charges. The forces that hold atoms together in molecules and which cause chemical reactions are all electrical. From this point of view, electrolytes differ from non-electrolytes in that certain of the charges bound up in their molecules can become comparatively free and therefore more reactive, chemically, when the electrolyte dissociates in solution. Electric charges are also bound up in non-electrolytes but in a more stable manner, so that the mere process of solution does not cause the disruption of molecules into ions. But the electronic structure of the atoms of non-electrolytes makes them subject to the influence of the free charges of the ions of electrolytes.

The Role of Solvents in Chemical Reactions.—Since ions bear a prominent part in chemical reactions, the solvent in which they are formed takes on a fundamental significance in chemistry.

Many striking examples of the failure of reactions to occur in the absence of a solvent are familiar to students of general chemistry. Hydrochloric acid gas and ammonia gas, when both are subjected to certain processes rendering them especially pure and absolutely dry, entirely fail to combine in spite of their usual great reactivity. Concentrated sulphuric acid and metallic sodium, two substances which in accordance with all our chemical experience seem about as certain to react with each other as would any imaginable combination, can be so thoroughly dried that the sodium when plunged beneath the surface of the acid shows no detectable reaction whatsoever. Of course the merest trace of moisture would start a violent reaction, but absolutely dry sulphuric acid and absolutely dry metallic sodium do not combine. Indeed, perfectly dry sulphuric acid does not even redden litmus paper, and in other ways fails to behave like an acid. These are just a few of the many surprising experiments which illustrate the significance of the solvent in chemical reactions. It has even been tentatively assumed that no chemical reaction can occur except under conditions which permit the presence of ions. We may at least assume that every chemical process including that of solution itself involves a rearrangement of electric charges such as exist upon ions.

The Physiological Significance of Water.—The preceding paragraph should make it already evident that water is no mere accidental or useless component of living matter. Henderson has admirably reviewed the various physicochemical properties of water which make it, of all known substances, the one solvent best adapted to further the peculiar group of

phenomena that we all life. Some of the most important properties may be briefly summarized.

The *solvent power* of water is remarkable. No other known liquid is able to dissolve so many and such diverse substances. Water is the only general solvent for inorganic salts. Although many organic compounds, for example fats, require organic liquids such as alcohol and ether for solvents, the majority of organic compounds are soluble in water. Among physiological substances, carbohydrates and proteins are more generally soluble in water than in any other liquid; while the list of substances, dissolved in blood and urine, includes scores of compounds of diverse character: Sugars, amino-acids, basic and acidic products of metabolism, gases, inorganic ions and many other substances. It has already been emphasized that protoplasm itself is a watery solution (p. 76).

Water is moreover *an electrolyte*. It does not dissociate to any great extent except under the influence of other substances and always the proportion of dissociated molecules to undissociated ones is very small; but even "conductivity water," which is purified to the greatest possible extent for use in experiments involving the measurement of electrical conductivity of solutions, contains at 22°C. approximately one ten millionth of a gram of hydrogen ions per liter of water. As the individual hydrogen ion is very light ($\frac{1.66}{10^{24}}$ g.), this weight of hydrogen ions represents about 60 million of them in 1 cc. of water; but even this large number represents a dissociation of only one in about 500 million molecules of water. As such water is perfectly neutral, the number of hydroxyl ions in it is equal to that of hydrogen ions. Since water dissociates to produce both H' and OH' ions¹ it is, in a certain sense, an amphoteric (amphoteric electrolyte). The importance of this property for hydrolytic processes has already been suggested (p. 21). Its significance in connection with hydrolytic dissociation of carbonates, phosphates and some other important physiological salts will be discussed later (p. 168).

Water not only dissociates itself, but has an especially high dissociating effect upon its solutes. Substances, that may be dissolved in various different liquids, are found to dissociate more completely in water than in most other solvents. Another way of expressing this fact is to say that water has a *high dielectric constant*.

Another significant property of water, mentioned above (p. 80) is its comparatively *high surface tension*; indeed, no other liquid except mer-

¹ Ions are written with symbols to distinguish them from non-ionic substances. Those carrying the positive charge are written with a dot, H'; and those bearing a negative one with a dash, OH'. The number of unit charges carried is indicated by the number of dots and dashes, Na', Ca'', Cl', SO₄'', PO₄''', which is equivalent to the valence of the ion. The signs H⁺ and OH⁻ are also used.

cury surpasses it in this respect. This property has undoubted value for protoplasm, enabling cellular structures to maintain their form, to produce protoplasmic membranes and, what is perhaps most important of all, to produce large and sudden changes in surface tension such as apparently occur in various types of protoplasmic movement.

Accompanying high surface tension is a *low internal friction* in water which results in a comparatively *low viscosity* of even such concentrated solutions as blood and cytoplasm. These properties favor the movements and the changes of form that occur in muscular contraction, amoeboid movement and other motile activities of living things.

A particularly valuable attribute of water is its great *heat capacity*. More heat is required to raise the temperature of water by a given amount than is required for any other known substance except liquid ammonia. Conversely, in cooling, water gives off more heat, per unit fall in temperature, than do other substances. No vital phenomena can occur except within a comparatively short range of temperature, covering some 60 or 70 degrees of the Centigrade scale; while many vertebrates must maintain a very nearly constant temperature. The heat capacity of water, acting as a buffer against sudden or extreme changes of temperature, is clearly a protective agency for living things.

Moreover, water, among all liquids, has the highest *conductivity for heat*. In this respect it even excels all solids except metals. This means that water is very useful in distributing heat in animals and plants and in the watery environment of many organisms.

An indication of the physiological importance of water is seen in its abundance in protoplasmic structures. The approximate proportions of water in some representative tissues are given in the following table:

Tissue	Percentage of water	Tissue	Percentage of water
Fatty tissue.....	6-10	Brain, white substance....	68-70
Bone (extremities and skull).....	14-22	Muscular tissue.....	75-78
Bone (vertebra and ribs).....	16-44	Thyroid gland.....	77-82
Tendon.....	56-68	Thymus.....	81
		Brain, gray substance....	82-85

Muscular and glandular tissues and gray matter of the brain contain a larger proportion of actively metabolizing protoplasm than do fatty and bony structures and tendons. The latter are storage and supporting tissues, largely composed of intercellular or other inert material. The less active structures are poorer in water content. Rate of growth and development are likewise correlated to water content. Embryonic tissues

contain a higher proportion of water than do adult tissues. Dried seeds and spores can grow and develop only when conditions are such as to permit them to take up water in adequate quantities.

Water also exerts important effects upon those terrestrial conditions which determine the character of the environment of living things. The value of the heat capacity of water in this connection has already been mentioned. The *latent heat* of water is equally important. By latent heat is meant the quantity required to change a substance from the solid to the liquid state or from the liquid to the gaseous condition without actually changing its temperature. It is equally well measured by the quantity of heat given out when the reverse changes occur. The latent heat of evaporation of water is greater than that of any other known substance. Consider what this means for the regulation and distribution of heat upon the surface of the earth. All bodies of water and more especially the oceans, since they constitute about $\frac{5}{7}$ of the earth's surface, absorb enormous amounts of the sun's heat without any such increase in the temperature as would occur in corresponding areas of rock or soil. This is partly due, of course, to the high heat capacity of water but largely to the latent heat. Water evaporates at all temperatures, even below 0°C . In this way it is constantly absorbing heat which is released again when the water condenses as fog or rain. Oceans are thus heat-distributing agencies. The freezing of water likewise liberates significant quantities of heat to serve as a further protection against low temperatures. Large bodies of water cannot take a temperature lower than their freezing point until the entire mass is frozen. We are naturally impressed by what seems to us great fluctuations in climatic temperatures; but if we could realize the much greater changes that would occur if the earth's surface were nearly waterless we would appreciate the fact that water goes a long way toward the conversion of our environment into a huge thermostat of limited range of temperature variation. For both animals and plants evaporation of water is an important means of getting rid of excessive heat. This will be more extensively treated in connection with the regulation of the temperature of the animal body.

The property of water, however, which in the face of all our other scientific experience is so unique as to seem weird, is the behavior of its density during changes of temperature over the range between 0°C . and 4°C . Most substances become continually more dense as their temperature decreases. Water, however, reaches a *maximum density* at 4°C . and with further fall of temperature down to 0°C ., it actually grows lighter so that ice is bound to float and the colder portions of any body of water near the freezing point will rise to the top. But for this property, bodies of water in freezing would begin at the bottom and freeze solid. Summer warmth would not necessarily melt them completely, and aquatic life would be greatly limited, except in the warmer zones of the earth.

Enough has been said to show that water has a combination of properties quite unlike those possessed by any other one substance. It is so familiar in our every day experience that we scarcely realize its significance. If water were as scarce as radium it might well excite an equally great scientific interest and wonderment. Certainly no other substance stands superlative or unique in so many physico-chemical properties. As Henderson has pointed out, an abundance of water at the surface of the earth is one of the things that make possible the existence of life with complexity of form and activity as we know it. It is conceivable that some form of life might exist by virtue of some other universal solvent, possibly liquid ammonia which has some peculiar and interesting properties; but given the chemical elements as we know them, we cannot find or even imagine any substance that would serve as well as does water for the solvent medium in protoplasm and as the regulator of the conditions of our environment.

The Cause of Osmotic Pressure and Its Measurement.—All substances in solution exert osmotic pressure; but the greater amount of the osmotic pressure in living things and in most of the fluids of animals and plants is dependent upon the presence of electrolytes. As osmotic pressure is the force that determines the movements of water across the many membranes of living structures; it has so important an effect on every type of vital activity that an attempt to understand its fundamental nature is worth while.

To begin with, one should recall the kinetic conception of matter which postulates, in accordance with the laws of thermodynamics, that all molecules are constantly in motion which could only cease at a temperature of absolute zero. As a corollary of this idea, we conceive of the molecules of matter in the fluid state as exhibiting not only vibratory motion but translatory motion as well. This results in the phenomenon of diffusion by which gases disperse evenly among each other, in so far as physical forces of gravitation and the like permit; while any solute dissolved in any solvent becomes evenly distributed throughout the entire solution, excepting in so far as surface tension (see p. 81) interferes. Suppose, however, that the solution is in contact with a semipermeable membrane, permeable to the molecules of the solvent but not to those of the solute. The conditions which then prevail are typified by the following specific instance. Suppose a dialyser is provided with a copper ferrocyanide membrane which is permeable to water but impermeable to sugar (Fig. 27). On one side of the membrane is a 10 per cent sugar solution and on the other is distilled water. Computing on the basis of the molecular weights of water (18) and sugar (342), we find that there are approximately 169 molecules of water in the solution to one of sugar. Molecular motion results in the continual bombardment of the membrane on both sides; but out of every 170 hits on the inside, 169 are made by water

molecules and one by a sugar molecule, while on the outside all hits would be made by water molecules alone. Since the membrane is permeable to water but not to sugar the chances are in favor of the passage of water from the outside to the inside of the membrane. The actually observed result is in accord with this conception, because the level of the solution inside the dialyzer rises. The solution then exerts a pressure measured by the difference in level between it and the water outside the membrane. This is osmotic pressure.

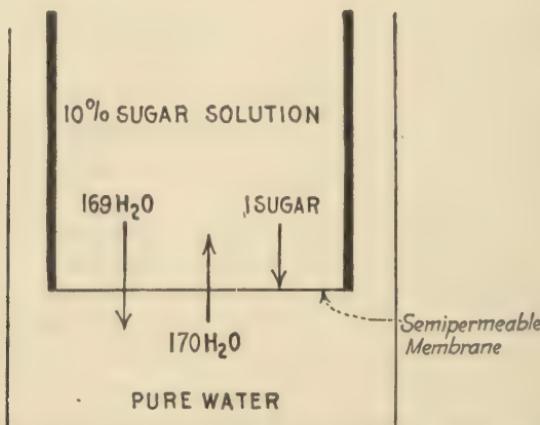


FIG. 27.—Diagram to show conditions which make osmotic pressure apparent. Explanation in text.

This explanation of the nature of osmotic pressure on the basis of thermodynamic theory is the classic one generally used since the first publication on the subject by Van't Hoff. It may be given equally well on the basis of the attractive forces between molecules, or the internal pressure of solutions. Either will stand the test of mathematical formulation or of experimental demonstration. This may perhaps seem surprising; but in the light of modern molecular physics it becomes intelligible, since both the degree of molecular vibration and the degree of molecular cohesion possessed by any substance are due, doubtless, to the same fundamental thing: The electronic structure of matter.

The incoming water dilutes the sugar solution so that its osmotic pressure is diminished; but theoretically, the sugar molecules, since they remain in the solution, would continue to exert a diminishing pressure up to infinite dilution which would be equivalent to distilled water and could never be attained. Actually, however, another force, gravity, intervenes and the pressure of the rising solution so increases the speed of the outward diffusion of water through the membrane that it eventually is able to balance the greater volume of inward diffusion and the whole process comes to an equilibrium. At this point the total osmotic pressure of the

solution is measured by the pressure exerted by the heightened level of the liquid within the membrane. If desired the solution within the membrane may be enclosed in an air tight cell connected to a bent tube containing mercury and constituting a mercury manometer (pressure measurer). The movement of mercury, since it is 13.6 times as heavy as water, will be much less before equilibrium is attained than would be the corresponding uplift of the solution itself. In this way comparatively little water need be taken in through the membrane. An apparatus for

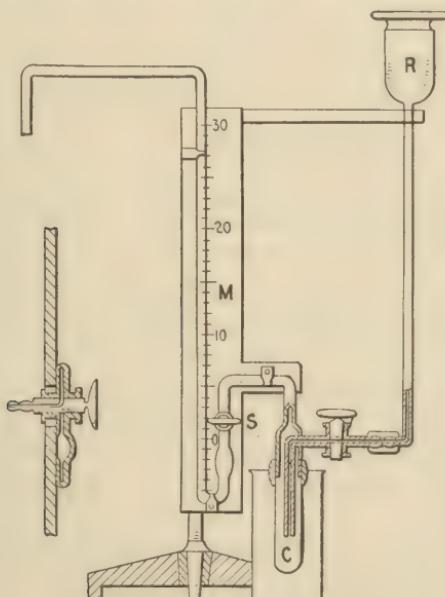


FIG. 28.—Osmometer. *C*, cup of porous clay upon which the semipermeable membrane is deposited. This cup is surrounded by a beaker of pure water. *R*, reservoir from which the solution to be tested is introduced into the cup. *S*, stop-cock through which the solution is brought in contact with the mercury of the manometer. A detail of the stop-cock is shown at the left. *M*, the manometer in which the pressures are measured. (After Hufner and Ganser).

direct measurement of osmotic pressure is called an osmometer. One type is shown in Fig. 28. The actual amounts of pressure obtained are surprisingly large. A 10 per cent cane sugar solution has an osmotic pressure of approximately 6.5 atmospheres. The term, osmotic pressure, grew out of the word osmosis which was applied by Dutrochet (1827) to the passage of water through a membrane, impermeable to some of the solutes in the water.

Van't Hoff's Law of Osmotic Pressure.—Since it is one of the forces which lift water from the soil to the tops of the highest trees and in other ways determine the movements of water within the plant, osmotic pressure quite naturally interested botanists. At any rate, it was a botanist, Pfeffer, (1877) who first made satisfactory quantitative measure-

ments of osmotic pressure on a sufficient number of concentrations of solution to permit deductions to be made. He found that, with cane sugar and other non-electrolytes, the osmotic pressure was proportional to the concentration and to the absolute temperature. From Pfeffer's results Van't Hoff (1885) was able to deduct the general theory that many substances in solution exert an osmotic pressure equal to the gas pressure that they would exert if their molecules were in a gaseous state and occupied a volume equal to that of the solution under the same conditions of temperature and pressure. Since a gram molecule¹ of gas occupies a volume of 22.4 liters at normal temperature and pressure, a force of 22.4 atmospheres would be required to compress² it to a volume of one liter. In other words the pressure of one gram molecule of gas occupying one liter is 22.4 atmospheres. One gram molecule of a solute in one liter of water, usually called a molecular solution, should exert, then, an osmotic pressure of 22.4 atmospheres at 0°C. and 76.0 cm. atmospheric pressure. In one of Pfeffer's measurements a 4 per cent sugar solution at 14°C. and 76 cm. atmospheric pressure gave an osmotic pressure of 208.2 cm. of mercury. To compare this result with the theoretical one, the measurement, as obtained, should be reduced to its value under standard conditions. At 0°C., supposing the gas law to apply, the pressure would be $208.2 \times \frac{273}{273 + 14} = 198.4$ cm. of mercury. Since a molecular solution of cane sugar ($C_{12}H_{22}O_{11}$) would contain 342 grams per liter, a 4 per cent solution is $\frac{40}{342}$ as concentrated as a molecular solution; so that, according to the theory, its osmotic pressure should be $\frac{40}{342} \times 22.4$ atmospheres or $\frac{40}{342} \times 22.4 \times 76 = 199$ cm. of mercury. A 1 per cent sugar solution gave an osmotic pressure of 52.1 cm. of mercury at 13.2°C. which means 49.5 cm. at 0°C., while the theoretical value is 49.7 cm. The results actually obtained, then, were in very close agreement with theory considering the experimental difficulties in getting accurate measurements.

Factors Modifying the Law.—Even as first formulated by Van't Hoff, the law was seen to be only a partial truth since it did not apply to comparatively concentrated solutions. In them, the attractive forces existing between the molecules of the solute and also those between the molecules of the solute and solvent, resulting in a sort of combination termed hydration or solvation, have to be taken into account in order to make observed osmotic pressures agree with theory. A description of

¹ A gram molecule, also called a mol, is as many grams as the molecular weight of the substance; for example, a mol of glucose, ($C_6H_{12}O_6$), molecular weight 180, is 180 grams.

² In accordance with the fundamental gas law of Boyle.

the operation of these forces is beyond the limits of this book. Works on Physical Chemistry may be consulted. Findlay's (1913) monograph on osmotic pressure may be found helpful.

Factors Affecting the Rate of Osmosis.—Van't Hoff's generalization is further limited in its application in that it only takes into account the final equilibrium, that is the total osmotic pressure of the solution. The rate of osmosis is markedly affected by circumstances, such as the electric charge on the membrane itself, to mention only one possibility. In the rapidly fluctuating conditions existing in and around living cells, any circumstances affecting the time relations of osmotic pressure, in addition to those altering final equilibria, would be of significance. Many of the factors modifying osmotic pressure are grouped together under the term *electric osmose*. Studies of these phenomena are still in a very partially developed stage but promise to yield results helpful in explanation of some curious anomalies of osmosis in living things. A few of these are: The working mechanism by which urine is secreted with a higher osmotic pressure than blood; the secretion of much watery material into the intestine after the injection of purgative salts into the blood or after their absorption from the intestine when ingested by the mouth in the usual way; the absorption of sugars, amino-acids, and salts by cells which appear to have a membrane impermeable to these materials.

Osmotic Pressure of Electrolytes as Shown by Plasmolysis Experiments.—Van't Hoff's law was only intended to apply to certain substances. It was seen to be obviously inapplicable to measurements of the osmotic pressures of electrolytes. We now conceive of their anomalous osmotic pressure and their conductivity as both due to the same fundamental property: Electrolytic dissociation; but at that time (1885) observations of these phenomena had not been extended sufficiently to permit such a generalization. Evidence was soon forthcoming. De Vries, like Pfeffer, a botanist, studied osmotic pressure in relation to living cells. He developed observations on the phenomenon called plasmolysis, protoplasmic shrinking. Under the microscope, plant cells, in layers sufficiently thin to permit observation or in slender filaments such as *Spirogyra*, are seen to have protoplasm completely filling the spaces within their comparatively rigid cellulose walls. If, however, the cells are immersed in a solution of high osmotic pressure, such as a 10 per cent sugar solution, the protoplasm is seen to shrink so as to draw away from the cell walls (Fig. 29). This is interpreted as signifying the withdrawal of water from it by the osmotic pressure of the solution which, though freely permeating through dead cellulose walls, finds the actual bounding membrane of the living protoplasm impermeable to sugar. The cell with a semipermeable membrane constitutes a natural osmometer. Many plant cells, after having been plasmolyzed, may be restored to their natural condition by returning them to water; so that the process is a

reversible one. DeVries found in plasmolysis a method for the quantitative measurement of osmotic pressures. He determined, in a series of observations, that particular concentration of sugar just sufficiently dilute to fail to plasmolyze. Such a solution was presumed to have the same osmotic pressure as the cytoplasm¹ and was called isosmotic or

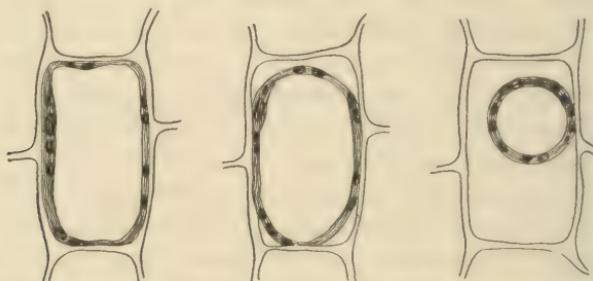


FIG. 29.—Plasmolysis. The cytoplasm, lying close to the cell wall in the normal cell at the left, draws away from the cell wall as plasmolysis proceeds.

isotonic. More concentrated ones may be termed hypertonic and less concentrated ones hypotonic. All solutions, isotonic to the same cells are regarded as having the same osmotic pressure. With *Spirogyra*, for example, certain substances are found to be isotonic at the concentrations given in the table below. When, taking their molecular weight into consideration, their molecular concentration is computed, they are seen to have approximately the same actual concentration.

Substance	Molecular weight	Percentage concentration which just fails to plasmolyze <i>Spirogyra</i>	Molecular concentration of the solution
Cane sugar.....	342	6.0	M 5.7
Glucose.....	180	3.3	M 5.5
Glycocoll.....	75	1.3	M 5.7

In short, solutions of each of these substances, having the same number of molecules per liter, show by plasmolysis the same osmotic pressure; just as they would by actual measurement in an osmometer. If, however, sodium chloride, or any other salt to which the cell is com-

¹ This is not necessarily true. Considerable evidence, now available, indicates that forces exerted by cell surfaces are capable of compensating for appreciable differences between the osmotic pressure of cytoplasm and that of the surrounding medium.

paratively impermeable, be used, a very different result is obtained. Plasmolysis occurs at a much lower molecular concentration. Potassium chloride, for example, plasmolyzes at $\frac{M}{10}$, but just fails to at $\frac{M}{10.03}$. The latter concentration must be isotonic with $\frac{M}{5.7}$ sugar or glycocoll.

The Electrolytic Dissociation Theory.—Many investigators, previous to DeVries, among them Pfeffer and Van't Hoff, had noticed the high osmotic pressure of solutions of electrolytes; but DeVries collected observations on a sufficient number of substances to enable Arrhenius (1887) to utilize the figures, together with results of conductivity and other measurements, in the formulation of his theory of electrolytic dissociation. This postulates that molecules of electrolytes in solution conducting the electric current need not be torn apart in order to produce the well known migration of anions to the anode and of cations to the cathode, but that the anions and cations actually exist in the solution at all times and are produced by the mere process of dissolving the electrolyte. Arrhenius further showed, from a comparison of known electrical conductivities of certain solutions with their observed osmotic pressures, that every ion in a solution exerts the same osmotic pressure that would be produced by a molecule. In the example cited above where $\frac{M}{5.7}$ sugar is isotonic with $\frac{M}{10.03}$ potassium chloride we find that the former is 1.81 times as concentrated as the latter, $(\frac{M}{5.7} \div \frac{M}{10.03} = 1.81)$. In other words, 100 molecules of potassium chloride exert as much osmotic pressure as 181 molecules of sugar. Conductivity measurements show that such a solution of potassium chloride contains, out of every 100 molecules, approximately 81 which are capable of conducting the current, that is, are dissociated into anions and cations. The agreement in this and many similar observations has been found to be so close as to fully establish the electrolytic dissociation theory and its application to osmotic pressure.

The ratio computed above between the concentration of a non-electrolyte and that of an electrolyte having the same osmotic pressure is called the *isotonic coefficient* (commonly represented by the symbol i) for the given solution of the electrolyte. It means that if the isotonic coefficient of a potassium chloride solution is 1.81, 81 per cent of its molecules are dissociated. In other words, out of every 100 molecules 81 have formed two ions so that there is a total of 181 particles, capable of exerting osmotic pressure. When substances dissociate to produce more than two ions, the computation is somewhat more complex; but the observations and their interpretations are entirely comparable. Salts of the type of Na_2SO_4 or $\text{Ca}(\text{NO}_3)_2$, dissociating to form three ions, are found

to have a still greater osmotic pressure in proportion to their concentration than have NaCl, KNO₃, etc.; but their isotonic coefficients can be measured and so their degree of dissociation reckoned. Such observations are also in agreement with conductivity measurements. The extent of dissociation (of which *i* is a measure) varies with temperature and dilution, increasing with both.

The far reaching effects of a theory, so scientifically fundamental as the idea of electrolytic dissociation, cannot be overestimated. Its influence on chemical science in general may be inferred from what was said earlier concerning the rôle of ions in reactions. In general physiology, it is essential to an explanation of the antagonistic action of salts and the physiological effects of salts as exerted by their individual ions. Its practical application is seen in a rational use of saline purgatives, of salt solutions for injection into the blood and for bathing of tissues, in determination of the inorganic requirements of crops, domestic animals and human beings. It is safe to say that there is no aspect of physiological science that has not been rendered more intelligible by the theory of electrolytic dissociation.

Especial Significance of Electrolytes in Bio-osmotic Pressures.— Since electrolytes give an osmotic pressure heightened in proportion to the number of ions produced by their dissociation, one reason for the above statement, that most of the osmotic pressure in living things is caused by electrolytes, becomes clear. Another reason is that electrolytes are, in general, of lower molecular weight and greater solubility than the non-electrolytes occurring in biological structures; so that a solution, that may be obtained under physiological conditions, will be of much greater molecular concentration in the case of an electrolyte than it will be in the case of a non-electrolyte. This may be true even though the two are present in the same percentage strength. An example will make this evident. The hemoglobin in the blood corpuscles constitutes about 31.7 per cent of their weight. This is one of the most concentrated protein solutions known; but since hemoglobin has a high molecular weight, given in one of the best estimates as 16,669, its molecular concentration is only $\frac{M}{52.6}$ and it has a correspondingly small osmotic pressure, such as would be given by a sodium chloride solution of approximately 0.06 per cent. Proteins would not seem to contribute much toward osmotic pressure. The same considerations apply to other colloidal constituents. Non-electrolytes of small molecular weight such as sugars, amino-acids and urea are capable of exerting considerable pressure; but none of them occur, in general, in large enough concentration in protoplasm or in plant and animal fluids to exert as much osmotic pressure as do the electrolytes. Sugar may be present in plant sap in sufficient concentration to produce high osmotic pressure and some other exceptions

are known; but in general, salts or at least electrolytes determine bio-osmotic pressures.

Other Methods for Osmotic Measurements—Cryoscopy.—It has been found valuable, in physiological investigations and in diagnosis of kidney diseases, to know the amount of osmotic pressure of blood, urine and other biological fluids. Determinations by direct measurement would involve experimental difficulties and tedious labor. Fortunately rapid methods, based upon similar principles, are available. One of them is determination of the boiling point, and another is determination of the freezing point. The temperature at which a liquid boils and that at which it freezes are changed by the presence of solutes in it. The elevation of the boiling point and the lowering of the freezing point are both proportional to the osmotic pressure. Boiling points are not very useful, however, since osmotic pressure may be changed by heating, which increases electrolytic dissociation, coagulates proteins and otherwise disturbs conditions in the solution. Freezing point determinations are of practical usefulness. The process is known as cryoscopy (cryoscopic method). A special thermometer of the differential type, usually named after its inventor, Beckmann, is required. It should distinguish differences of temperature as small as 0.01°C . and still finer distinctions are desirable. The apparatus with thermometer is shown in Fig. 30. Since the difference between the freezing point of water and that of a watery solution is directly proportional to the osmotic pressure of the solution, so that all watery solutions showing the same freezing point have the same osmotic pressure, the lowering of the freezing point of water by the solutes becomes a complete measure of the osmotic pressure of the solution. It need not even be translated into an expression of actual pressure when, as is usually the case, only comparative data are required. The result is denoted by the symbol Δ . Example: A specimen of blood freezes at $-0.602^{\circ}\text{C}.$, and as the freezing point of water is taken to be $0^{\circ}\text{C}.$, $\Delta = 0.602$.

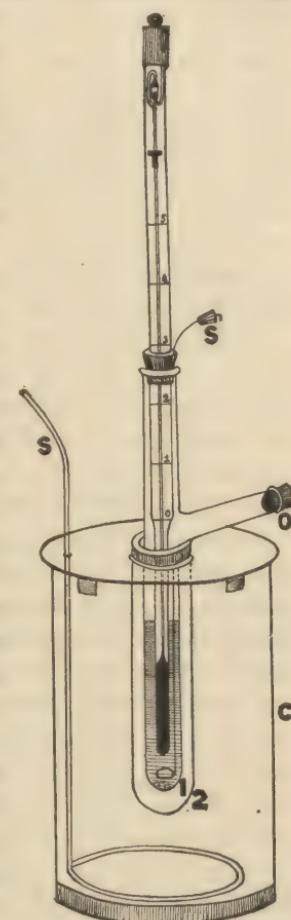


FIG. 30.—Apparatus for determination of the freezing point. *C*, container for freezing mixture of ice and salt. 1, test tube containing the substance to be tested. 2, large test tube to serve as a jacket. *O*, opening for introduction of liquid to be tested. *S*, *S*, stirring wires. *T*, delicate thermometer of which the fine graduations are not shown.

The quantitative relationship between osmotic pressure and Δ is such that a decimolar ($\frac{M}{10}$) solution of a non-electrolyte lowers the freezing point 0.185°C . The actual osmotic pressure of such a solution is 2.24 atmospheres or 170 cm. of mercury (76×2.24). An $\frac{M}{11}$ solution freezes at -0.168°C . and has an osmotic pressure of 154.5 cm. of mercury. These figures show that a difference of 0.001°C . in the freezing point corresponds to a difference of 9 mm. of mercury. It is difficult to determine Δ with an error not exceeding 0.001°C ., but pressures differing from each other by as much as 9 mm. of mercury are easily distinguished. Cryoscopy, then, is a less sensitive method than osmometry, but its greater convenience has made it preferable for most determinations.

Osmotic Pressures of Some Biological Fluids.—The actual values obtained for some biological fluids are of interest. Urine shows wide variations. The freezing point of human urine, after copious water drinking, may be as high as -0.2°C ; after water deprivation and comparatively high salt intake, it may be below -3.0°C . This illustrates the marked tendency of the kidneys to protect the body from suffering changes in the osmotic pressure of the blood. The latter, for mammals and indeed for most vertebrates, is comparatively constant. For human blood Δ is 0.56 (average) and variations lie within the limits 0.51–0.62 under physiological conditions. Greater variations indicate disorder, usually of the kidneys. The osmotic pressures of the bloods of different mammals are remarkably similar:

Animal	Δ (average)	Animal	Δ (average)
Man.....	0.560	Rabbit.....	0.592
Ox.....	0.585	Dog.....	0.571
Horse.....	0.564	Cat.....	0.638
Pig.....	0.615	Sheep.....	0.619

These figures are taken from results obtained by various observers. If an equally large number of determinations were made on the blood of each species under the same variations of conditions, a still closer correspondence would possibly be seen. Among other vertebrates, birds and some reptiles have about the same osmotic pressure of blood as have mammals.

Comparative Power for Regulation of Osmotic Pressure in Animals.—Fishes show a greater variation. The elasmobranchs, or cartilaginous fishes, may be regarded as low in the scale of evolution of the vertebrates. Their blood has approximately the same osmotic pressure

as sea water. For the blood of sharks and other elasmobranchs investigated, Δ varies between 2.25 and 2.45, while for sea water it is 2.30 (average). Marine bony fishes, regarded as higher in the scale of evolution, have a lower osmotic pressure of the blood, ($\Delta = 0.85$ to 1.05) which they maintain in spite of the surrounding sea water; while the whale, a marine mammal, has blood of the same osmotic pressure as land mammals. Marine invertebrates, on the other hand, show an osmotic pressure of their body fluids equal to that of sea water. Moreover when they are transferred to diluted or concentrated sea water their body fluids change accordingly. This is shown by the following figures from an experiment by Fredricq:

	Δ of sea water	Δ of body fluid of the Crustacean, Maia
Normal sea water.....	2.30	
Concentrated sea water.....	2.96	2.94
Diluted sea water.....	1.38	1.40

The blood of cartilagenous fishes shows a similar tendency to vary with the surrounding water; but the anadromous fishes, which leave the deep sea and go up into fresh water during the breeding season, only very slightly modify the osmotic pressure of the blood. In general, the more specialized the animal form, the more perfectly is it adapted to regulate the osmotic pressure of its fluids.

Isotonic Salt Solutions.—Many of the cells of higher animals are so constructed that their very existence is dependent on the maintenance of the osmotic pressure of the surrounding medium within very narrow limits of variation. Red corpuscles of mammalian blood are especially convenient cells for demonstration of the disastrous effects of changes in osmotic pressure. If immersed in 0.9 per cent NaCl solution, which has approximately the same osmotic pressure as blood and is therefore called isotonic or physiological salt solution, corpuscles appear to be unchanged; but in any solution of lower osmotic pressure, they swell to a larger volume. This may be demonstrated by the use of the hematocrit, a centrifuge tube, in which a given amount of blood, mixed with a solution under investigation, may be whirled at high speed so that corpuscles will be packed at the bottom where the tube has a narrow bore and fine gradations. This permits determination of the volume of the corpuscles after centrifugation. The hematocrit demonstrates swelling of corpuscles in solutions of osmotic pressure lower than that of blood and shrinking in solutions of higher osmotic pressure. Water is taken in or given off by the corpuscles across their stroma membranes (see p. 74) in accor-

dance with changes in the osmotic pressure of the surrounding medium. Any solution of osmotic pressure less than that of a 0.9 per cent NaCl solution (isotonic) is termed hypotonic and those of higher osmotic pressure are called hypertonic. In addition to volume changes, some corpuscles in a hypotonic solution suffer actual disruption so that the

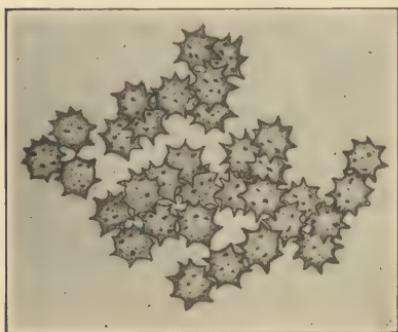


FIG. 31.—Crenated corpuscles. Showing the effect of exposure to solutions of high osmotic pressure.

hemoglobin of their contents is dissolved out and imparts its red color to the solution. The more hypotonic the solution, the more rapidly does hemolysis (destruction of blood cells) occur. In water, corpuscles are destroyed almost instantly. The phenomenon has been described above (p. 75 and Fig. 19). The opposite effect, shrinkage in hypertonic solution, results in what are called crenated corpuscles whose microscopic appearance is shown in Fig. 31.

Effects of osmotic pressure changes, though easily made visible in the

case of blood corpuscles, are not confined to them. One has only to recall the common experience of the irritating sensation in a cut, when it is exposed to either fresh or salt water, to realize that pain nerves are in some way sensitized to register the effects of osmotic pressure changes. Physiological salt solutions do not have irritating effects when in contact with open wounds or such delicate mucus membranes as those lining the turbinal spaces of the nose. This fact is the basis of the common surgical practice of using only physiological saline in bathing exposed tissues. Any solutions injected into the blood stream, as in cases of severe hemorrhage, must obviously possess an approximately correct osmotic pressure to avoid serious hemolysis. So far as mere osmotic pressure is concerned, solutions of cane sugar, glucose, urea or any one of a number of other non-electrolytes may be made in the correct concentration for any type of animal or plant cell so as to appear osmotically neutral. Of cane sugar, a 10 per cent solution (0.32 molar) is isotonic for mammalian cells. All such solutions, however, have more or less toxic effects, if for no other reason than that they cause diffusion of electrolytes away from the surface of the cells.

Balanced Isotonic Solutions.—It is doubtless already clear to the reader that osmotic pressure of a cell medium is not the only attribute requiring physiological regulation. The kinds of electrolytes and the proper balance of their concentrations are fundamentally important. The first extended investigations of the comparative effects of different salt solutions upon living animal tissues were made by Ringer (1880—

1883). It had long been known that pure NaCl solution was comparatively harmless to living tissues and appeared to further some, at least, of their activities. An excised frog heart in 0.7 per cent NaCl solution (isotonic with frog blood) continues to beat rhythmically; but, as Ringer showed, the force of its muscular contraction diminishes rapidly; so that in the course of an hour or so, it dies away to a merely detectable pulsation in a small portion of the muscle. Eventually it ceases altogether. Ringer found that if calcium chloride were added to the sodium chloride solution the beats could be made to resume, though an excess of calcium would bring the heart to a standstill in a contracted condition (heart rigor). He also found that potassium chloride added to the solution abolished the rigor; so that, with all three salts in the medium, normal heart beats were obtained. Excess of potassium or an isotonic solution of pure KCl caused stoppage in an entirely relaxed condition, (inhibition). From the known proportions of sodium, potassium and calcium in blood, Ringer devised a solution capable of maintaining the beat of the frog heart for an indefinite period. In addition to the chlorides of sodium, potassium and calcium, it contained sodium bicarbonate which was used to imitate the slight alkalinity present in blood. The significance of this constituent in maintaining a reaction very close to neutral will be discussed below. Ringer tried the substitution of other salts of calcium and potassium, such as the carbonates, sulphates and phosphates, for the chlorides and found that, in the presence of sodium chloride, they produced the same effects irrespective of the anions. He proved, in short, that it was calcium and potassium cations themselves which caused, respectively, heart rigor and heart relaxation. This is interesting in view of the fact that the electrolytic dissociation theory was unknown at the time of his work. The Ringer solution for the frog heart has the following composition:

	GRAMS		GRAMS
NaCl.....	6.5	NaHCO ₃	0.20
KCl.....	0.14	Water.....	1,000.00
CaCl ₂	0.12		

Glucose, in concentration between 0.1 and 0.2 per cent, is an additional advantage, since it serves as fuel to supplement or to spare the store of energy-producing material in the heart muscle. Glucose is required in a solution used to maintain the beat of the homoiothermal ("warm-blooded") heart, as it does not contain so large a carbohydrate store as do the muscular tissues of poikilothermic ("cold-blooded") animals.

To correspond to the higher osmotic pressure of bloods, salts must be present in slightly greater concentration in an artificial medium for the mammal or bird heart. Locke devised a solution adequate for maintain-

ing the excised heart of dogs, cats and similar laboratory animals. He recommended (1900) the following composition:

	GRAMS		GRAMS
NaCl.....	9.0	NaHCO ₃	0.20
KCl.....	0.42	Glucose.....	1.0-2.5
CaCl ₂	0.24	Water.....	1,000.0

A solution of this or similar composition may be termed a Ringer-Locke solution.

An abundant supply of oxygen is also required; for although the frog heart can obtain enough oxygen in the form of that dissolved from the air in the Ringer solution, the warm-blooded heart, to maintain its beat in artificial surroundings, must have a constant stream of oxygen gas bubbling through the solution.

Recalling the well known constancy of temperature of most mammals, it is to be expected that a mammalian heart, when excised, can maintain activity only if kept in a thermostat at body temperature (37-38°C.). Such is the case.

If from a dog, under anaesthesia, the heart is removed while still beating and quickly immersed in oxygenated Ringer-Locke solution at body temperature, beats will be maintained in regular rhythm for considerable periods—even days. Disturbing conditions will eventually intervene. Bacterial infection would, of course, destroy the tissues. This difficulty may be overcome by careful sterilization of the solution and the use of extreme aseptic surgery in removal of the heart. Even so, however, tissues at the cut ends of severed blood vessels are bound to die; so that products of their disintegration in time affect the other tissues of the heart. This is an instance of a principle of general physiological application and of practical significance. Autolysis (self destruction) of dead tissues always produces substances that are toxic for living cells. This is probably the fundamental cause of death from wound shock which took so sad a toll of life during the great war. Moreover, electrolytes, even when supplemented by sugar and oxygen to maintain oxidation, are not the only requirements of heart muscle. Experiments with excised cold-blooded hearts have shown that frequent changes of Ringer solution remove something, the loss of which causes a gradual decline in the force of the heart beats. There is some evidence to show that the necessary constituent is of a lipoid character but proof is incomplete. At any rate, hearts, nearly quiescent from the lack of it, may be restored to practically normal activity by the addition to the surrounding medium of a small proportion of Ringer solution which has been allowed to repeatedly circulate (perfuse) through the chambers of another excised heart. The longest period of successful maintenance of activity in an excised heart is, so far as the writer is aware, 38 days. A frog heart in

oxygenated, sterile, sugar-containing, Ringer solution maintained its rhythm at a rate which scarcely diminished until the end of this extended period.

Practical Use of Physiological Salt Solutions.—One practical application of knowledge concerning physiological salt solutions has been the development of a technique by which living tissues can be temporarily preserved outside of the body. This makes them available for surgical transplantation into human beings suffering from a deficiency of certain essential structures. Persons in normal health, killed by accident, have of course entirely healthy tissues. Small portions or even whole organs may be removed aseptically, kept in a suitable sterile solution and later transplanted into other human beings. The period of preservation outside the body is usually from 24 to 48 hours and some tissues may be preserved for still longer times. Some types of cells cannot survive this treatment but successful transplantations of sections of artery have been made. Testes and ovaries have been transplanted whole and although subsequent reproductive functions have not been possible in mammalian transplants, yet some of the structures in these organs continue to function. They undoubtedly give off what are called internal secretions into the blood. Effects of these products, coming from transplanted organs, have been noted in the form of altered secondary sex characters, growth of hair, changed sex behavior and the like, also in effects on metabolism and general bodily appearance. These and some other more or less successful transplantations would not have been possible but for our knowledge of physiological salt solutions.

The maintainance of a proper volume of blood in the body has also become possible through the injection of salt solutions. A Ringer-Locke solution or a mere 0.9 per cent NaCl solution may be used to replace blood lost in severe hemorrhage. In some cases, however, large quantities of salt solution fail to keep the blood vessels properly filled. Even repeated injections are followed by loss of blood pressure. This was a serious difficulty encountered in many cases of wound shock during the Great War. The explanation most commonly accepted is that toxins given off by seriously wounded tissues produce, among other effects, an altered permeability of the walls of blood vessels, particularly the capillaries. As a result of this, salts which ordinarily would not diffuse out of the blood stream into the surrounding tissues to any significant extent, are now rapidly lost from the blood. The osmotic pressure of the blood is therefore lowered sufficiently to make it unable to hold the usual amount of water in the blood stream. To overcome the difficulty, Bayliss devised a solution which was used with surprising success in many cases of wound shock. The essential principle utilized was the extreme impermeability of membranes to colloids. It would seem that if a solution containing a large quantity of colloidal material could be infused into

the circulatory system, the rapid loss of fluid from the capillaries into the surrounding tissues would be checked, since the colloid material would stay in the blood stream and maintain the requisite osmotic pressure. After various trials with a number of substances, Bayliss and other workers associated with him in war-time investigations, hit upon gum arabic as a suitable material. This substance was described in Chap. IV as a complex polysaccharide composed chiefly of pentosans. In animal bodies it is only very slowly changed and appears to be without specific physiological effects. Commercial gum arabic contains potassium and calcium in some unknown combination. It was found, therefore, unnecessary to use potassium or calcium other than that in gum arabic which was merely dissolved in isotonic sodium chloride solution.

It will be recalled from Chap. V that sodium, potassium, calcium and magnesium very generally occur in living tissues and animal and plant fluids. Since only three of these appear in Ringer and similar solutions, one naturally wonders if magnesium is not a necessary constituent. So far as effects on the heart are concerned, it seems to be a matter of indifference as to whether or not magnesium be present. In other observations, however, magnesium is shown to be definitely useful to certain tissues. A loop of cat or other mammalian intestine if immersed in Ringer-Locke solution at body temperature will exhibit slow contractions which occur in regular rhythm, constituting slow, pulsating beats. They soon die out. If, however, magnesium salts, in suitable concentration, are added to the solution the intestine resumes its beating which continues many hours. Tyrode (1910) devised a solution for this purpose using magnesium chloride and mono-sodium phosphate in addition to the usual salts. His formula is as follows:

	GRAMS		GRAMS
NaCl.....	8.0	NaH ₂ PO ₄	0.05
KCl.....	0.2	NaHCO ₃	1.0
CaCl ₂	0.2	Glucose.....	1.0
MgCl ₂	0.1	Water.....	1,000.0

Similarity of Blood Salts to Sea Salts.—Macallum (1903), discussing the theory that life originated in the sea, called attention to the fact that the relative concentrations of sodium, potassium, calcium and magnesium in the blood of all vertebrates are nearly identical with and are in all cases very similar to the proportions found in sea water. The actual concentrations of the salts are less than in sea water and one constituent, magnesium, is both actually and proportionally less. In certain marine invertebrates, on the other hand, the body fluids contain these ions in almost the same actual concentration in which they occur in sea water. Some illustrative figures, as presented by Robertson from Macallum's data, are given below. In each case the percentage concentration of

sodium is taken as 100 and the percentage of the others are reduced to proportional figures.

Substance	Na	K	Ca	Mg
Sea water.....	100	3.6	3.9	12.1
Tissue fluid of a jelly fish.....	100	5.2	4.1	11.4
Serum of the lobster.....	100	3.7	4.9	1.7
Blood serum of a dog.....	100	6.9	2.5	0.8

It is seen that the jelly fish, regarded as representative of the lower marine invertebrates, compares very closely with sea water and the lobster shows a proportion of Na to Mg intermediate, as regards its tendency to resemble sea water, between the jelly fish and the mammal. Macallum interprets these and a mass of similar data as signifying that an animal, not possessed of a kidney or homologous organ, cannot accurately regulate the concentration of inorganic salts in its fluids, but must allow them to assume approximately that of the surrounding medium. In those forms which, like the lobster, possess a kidney for regulation of blood composition, the salts may be maintained in proportions that are independent of the environment. The actual concentrations found in the blood of the different vertebrate species reflect, according to Macallum's theory, the concentrations prevailing in the sea water during the geological epoch in which their phylogenetic ancestors first acquired the regulative power. The primitive kidney, arising in the protovertebrate forms from which the vertebrates descended, might readily have been adapted to the concentrations of salts in their body fluids and may have transmitted the same adjustments to their descendants down through the ages.

To account for the differences between the concentrations of salts in vertebrate blood and in sea water, Macallum suggests the very plausible idea that during the early Cambrian period when land forms are supposed to have arisen, the ocean had a composition distinctly different from that at the present time. It might well have been less concentrated then than now. Salts are constantly being brought to it by rivers and though the concentration would not perceptably change during human history, it would decidedly increase during geological ages. This would account for the differences in actual concentration. The discrepancy in relative concentration of magnesium is very marked, however. This might be explained by the fact that, while potassium and calcium are being constantly removed from sea water by growth of animal and plant forms, magnesium is not so largely utilized. Calcium moreover is permanently removed since enormous deposits of diatom and other shells, coral reefs,

etc. are constantly forming under the sea. Potassium, utilized in such large proportion by land plants, would not reach the sea in quantities proportionate to that dissolved by land erosion. Magnesium salts, however, would be freely carried oceanward and would continue to accumulate, so that while sodium, potassium, and calcium increased slowly but without marked change in proportions magnesium would increase relatively faster. Macallum's theory seems, on the whole, well founded and certainly the resemblance of the salt content of the blood of widely differing species of animals to that of sea water is striking.

It is of further significance that sea water, so diluted with distilled water that it is isotonic with blood, constitutes a physiological salt solution suitable for many uses. It still contains, however, too high a concentration of magnesium. The latter is sufficient, when sterilized isotonic sea water is injected into the veins of rabbits, to cause the type of glycosuria (mentioned in Chap. V.) produced by administration of pure magnesium salts.

Physiological Effects of Specific Ions.—That the several metallic ions have different physiological effects has already been suggested in several connections.

Sodium appears to have a comparatively mild effect on tissues. Although it antagonizes the effects of calcium it is not as effective in this respect as potassium. In general, sodium ions have been regarded as chiefly useful in maintaining osmotic pressure. This view seems inadequate since isotonic NaCl solution and isotonic sugar solution are not equally effective in maintaining contractile power of heart or other muscular tissues. The comparative scarcity of sodium inside of cells, that is, in cytoplasm itself is a striking fact. Fahr kept frog muscles immersed in isotonic sugar solution to permit salts of the blood and lymph to diffuse away and then found that analysis of the muscle revealed practically no sodium but a supply of potassium only very slightly less than that normally found in muscle. His results are given below:

Material used for analysis	Results of analysis—per cent of muscle weights		
	Total ash	Sodium	Potassium
Fresh sartorius muscle of frog.....	1.036	0.066	0.34
Same after 6 hours in isotonic sugar solution..	0.790	0.005	0.28
Same after 20 hours in isotonic sugar solution..	0.787	0.009	0.24
Same after 20 hours in Ringer solution.....	1.058	0.069	0.31
Frog blood serum.....	0.699	0.241	0.13

Whatever effects sodium may have upon living cells must be largely exerted upon the exterior.

The stimulating effect of Ca^{++} ions and the inhibiting action of K^{+} ions on the heart were described above. Similar effects upon skeletal muscle have been demonstrated. When Ca^{++} or K^{+} ions are present in excess of physiological concentration in a solution artificially circulated (perfused) through the legs of a frog, the muscles soon become non-irritable, that is, they do not twitch in response to an electric shock. They recover irritability if the unfavorable medium is replaced by Ringer solution. Many similar instances of marked changes in physiological behavior due to alteration in the concentration of one or more ions are on record. It seems impossible to summarize such data by labeling each ion as the one which always plays the same rôle. They have various effects under different conditions and in different tissues.

Whatever their specific effects may be, however, the interactions of Na^{+} , K^{+} , Ca^{++} and Mg^{++} ions in living things take an essential part in vital activities. This has been especially demonstrated in animal experimentation. But there is evidence that it applies to all protoplasm. In vertebrates, the concentrations of these ions and their relative proportions in tissues and fluids are of such fundamental importance that comparatively slight alterations produce profound changes in physiological behavior.

Negative ions are also of physiological significance. The chief anions furnished by inorganic salts of living things are: Cl^{-} , CO_3^{--} , HCO_3^- , $\text{PO}_4^{'''}$, $\text{HPO}_4^{''}$, $\text{H}_2\text{PO}_4'$ and $\text{SO}_4^{''}$. A fundamental reason for their presence is that electrolytic dissociation in solutions must produce anions and cations bearing equivalent charges. If the resulting electrical neutrality should tend to be altered by any event, a further compensatory dissociation must immediately occur in such manner as to restore an electrical balance. It is a basic concept of electromagnetic theory that any tendency to accumulate a positive charge is immediately followed by the production of an equal negative charge in the near vicinity. This is seen in the phenomena of induced charges in an electrical condenser or what in nature amounts to the same thing on a huge scale—the charging of thunder clouds. Any accumulation of charge on one cloud induces an equal and opposite charge on another or upon the earth until discharge occurs in the lightning flash. The presence of any positively charged ions presupposes, then, the presence of anions sufficient to carry an equal negative charge. It is, indeed, the movements of these positive and negative charges in and through living matter which constitute the electrical phenomena of physiology to be described later in connection with discussions of the phenomena of nerve, muscle and gland activities. No physiological event occurs without an accompanying electrical disturbance; as we ought perhaps to expect, if we believe that no chemical event takes place without action of ions.

Specific effects of individual anions corresponding to those of cations have not been clearly shown; but here again, the balance of their concentrations is significant. Elimination of CO_3'' , PO_4''' , SO_4'' and Cl' ions, not to mention other less abundant anions, is so adjusted to their production in the body, that a nearly uniform concentration of each is maintained in the blood. Similar regulations affect all biological fluids including protoplasm itself. These facts suggest that the different anions are not identical in physiological value. One instance of specific activity of anions is due to cell permeability. The Cl' and CO_3'' ions pass in and out of cells more readily, at least more rapidly, than do other anions; so that they are especially concerned in the regulation of concentration of ions in living matter and the surrounding medium. CO_2 is a product of oxidations constantly proceeding in all protoplasm at a rate, varying in proportion to physiological activity. CO_2 , reacting with water, produces the ions H' and HCO_3' . Chlorides are the most abundant salts in all animal organisms. HCO_3' and Cl' , then, are the most abundant physiological anions and as cells are permeable to them, they are able, by passing in and out of cells, to preserve the proper balance of anions in relation to cations within protoplasm.

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CHAPTER VII

THE SIGNIFICANCE OF HYDROGEN AND HYDROXYL IONS IN PHYSIOLOGY. THE REGULATION OF NEUTRALITY IN THE ORGANISM. DYNAMIC EQUILIBRIA

Of the many sorts of ions resulting from electrolytic dissociation in living matter, hydrogen and hydroxyl ions are worthy of especial study because they take part in or influence so many of the reactions—perhaps all of the reactions that occur in living matter. This is, of course, one way of saying that changes, producing acidity or alkalinity, have very marked effects on living matter.

Sensitiveness of Living Things to H' and OH' Ions.—The fatal effects of strong acids and alkalies are familiar enough. The toxic effects of comparatively small changes in reaction are not so obvious. Let us take an example from physiological experimentation. If blood or an artificial medium, circulating through a frog heart, changes from its physiological condition of approximate neutrality to one of faint but detectable acidity, the heart is killed. The fatal change is, however, so small that the same degree of acidity would be produced if 1 cc. of 0.004 per cent hydrochloric acid were added to one liter of neutral distilled water. The resulting 0.000004 per cent HCl solution would not even taste sour and would produce so slight a color change in delicate litmus paper as to require care in its detection. The weight of hydrogen ions in such a solution is in the proportion of one part to about one billion parts of water. This seems a very small change to produce so striking an effect; but changes in the concentration of hydrogen ions amounting to less than one tenth of this magnitude may produce profound, even if not fatal, effects on many living cells. It is clear that ordinary methods for measurement of acidity are not sufficiently delicate to detect the differences that are of physiological significance. Before describing the methods that are sufficiently delicate, it will be necessary, however, to review the modern conventions used to express the concentration of hydrogen ions.

Normal Solutions.—It is customary in quantitative chemistry to express concentrations in terms of normal solutions. A normal solution is one which contains as many grams of the substance dissolved in one liter of the solution as the combining weight of the substance. In some cases the combining weight is identical with the molecular weight. For HCl and NaOH, which combine in equimolecular quantities, their

molecular solutions are the normal ones, namely, 3.645 per cent and 4.005 per cent respectively. They are written N HCl and N NaOH. With H₂SO₄, however, one molecule requires two of NaOH for neutralization; so that its combining weight is one-half its molecular weight. Its normal solution, then, is 4.903 per cent. The combining weight of a substance may be defined as the weight in grams which will combine with, replace or oxidize one gram of hydrogen. The advantages of the use of normal solutions may already be familiar to the reader, but are worth recalling. Equal volumes of any two normal solutions which react with each other are chemically equivalent; so that 10cc. of any normal acid are exactly neutralized by 10cc. of any normal alkali. Similarly, the silver of 10cc. of normal AgNO₃ would be completely precipitated by 10cc. of normal NaCl. Normal solutions greatly simplify many chemical calculations. Moreover, the relative strengths of solutions are much more apparent in terms of normality than in percentages. Thus one sees at once that $\frac{N}{10}$ HCl and $\frac{N}{20}$ H₂SO₄ (also written 0.1N and 0.05N) are of such strengths that the former requires an equal volume of $\frac{N}{10}$ NaOH to neutralize it, while the latter needs only half as much; but such relations would not be clear if the same concentrations were given as: 0.364 per cent HCl, 0.245 per cent H₂SO₄ and 0.200 per cent NaOH.

Normality of Ion Concentrations.—It is equally convenient to use normalities to express the concentration of ions. We must lay particular stress, however, upon the fact that normality of ions in a solution cannot be inferred from mere inspection of the figures for normality of the solute that furnishes the ions. Thus 0.1 N NaCl is not 0.1 N as regards Na⁺ and Cl⁻ ions; since, as explained in describing the isotonic coefficient, only about 84 per cent of the NaCl molecules effect electrolytic dissociation in the solution. Different electrolytes have very different powers of dissociation. This becomes particularly evident, upon examination of a series of acids or bases. The essential difference between the so-called "strong" acids and the so-called "weak" ones is merely in the degree of dissociation. HCl is called a strong acid because in 0.1 N concentration it is about 90 per cent dissociated while acetic acid is regarded as comparatively weak since its 0.1 N solution is only about 1.3 per cent dissociated. According to normalities the two solutions are of equal strength and would require the same amounts of alkali to neutralize them; but according to real acidity (the actual abundance of H⁺ ions), one is about 70 times as strong as the other. Similar contrasts may be found among alkaline solutions. 0.1 N NaOH and 0.1 N Ca(OH)₂ are neutralized by the same quantities of acid but the former dissociates so that a large majority of its molecules produce OH⁻ ions, while the

latter is very weakly basic and has only a small proportion of its molecules dissociated.

The Significance of Titration.—The method for determination of acidity and alkalinity by ordinary chemical analysis is titration. What does titration show us? Let us take an example. We desire to know the reaction of a specimen of urine. Having tested with litmus paper to find that it is acid, we put 100 cc. of it in a beaker, add carefully standardized 0.1N NaOH from a measuring burette and observe how much is required to change the color of some indicator substance previously added to the urine. It will make a considerable difference what indicator is used. More alkali will be required to neutralize to phenol phthalein than to phenol red. Suppose we use phenol red because that has been shown by methods, to be described presently, to change from the yellow color which it shows in acid solutions to a brilliant red at the time when the solution is very close to neutral, that is, when it has no preponderance of either H' or OH' ions. Having observed carefully that enough alkali was added to bring the urine to a stage of neutrality, shown by the proper tint of the indicator, suppose we find that the 100 cc. of urine has required 50 cc. of $\frac{N}{10}$ NaOH. The result of titration makes it appear that this specimen of urine is $\frac{N}{20}$ acid. Is this an index of a correspondingly high concentration of H' ions? Most certainly not. If urine were 0.05 N in H' ions it would give a burning irritation to the lining of the urinary passages and would otherwise work serious havoc with the body that secreted it. The high titration value represents the total concentration of urinary substances capable of yielding H' ions to a solution. All of them do not dissociate at any one time. Each acid substance can only slightly dissociate. Each of them, however, reaches an equilibrium between the forces tending to make it dissociate into ions and those tending to keep it in the form of undissociated molecules. The reaction may be represented thus:



where HA stands for any substance capable of furnishing the H' ion together with some acidic ion represented as A'. The equilibrium is not a static one, but, as the oppositely pointed arrows indicate, represents a balance between forces driving the reaction from left to right and those forcing it from right to left. Anything which disturbs this balance will cause a compensatory reaction. This is merely an application of the fundamental law of mass action. When OH' ions in the form of NaOH solution are added to urine they combine with the H' ions to form water which is not dissociated. Consequently the active mass of H' ions

tends to diminish. It does not actually diminish, however, as long as more HA is present to maintain the reaction



As fast as more NaOH is added more HA dissociates until it has all been dissociated so that the solution, by addition of a little more alkali, may be neutralized. Although the total number of acid-forming molecules was sufficient to show a titration value of $\frac{N}{20}$ acid, the actual concentration of hydrogen ions, which is after all the true acidity of a solution, may not have been, even before any alkali was added, any more than $\frac{N}{100,000}$.

This example, showing the limitations of the use of titration, should not be interpreted as signifying that it is valueless. It shows the total amount of acidic substances in liquids. In biochemical work, this information is sometimes valuable. In quantitative analysis, it is oftentimes the only information required. Wrongly interpreted, however, titration values may be very misleading. A famous instance of this is seen in the older statements concerning the reaction of blood. Titrated, it appeared to be quite alkaline; so that descriptions of blood, written before 1908,¹ called it alkaline. In terms of actual concentration of OH' ions, however, it is so close to neutral that if a perfectly neutral solution, which always contains a small but equal number of H' and OH' ions, be represented by

$\frac{N}{10,000,000}$ OH', a specimen of blood might be $\frac{1.6N}{10,000,000}$ OH'. Ordinary tap water is usually more alkaline than blood although to casual observations or to titration it appears to be neutral. Distilled water as commonly prepared in the laboratory has a greater concentration in H' ions than blood has in OH' ions. Instead of calling blood alkaline we might better speak of it as nearly neutral or just on the alkaline side of neutral. Only by a statement of concentration of hydrogen or hydroxyl ions do we describe the true acidity or alkalinity of a solution.

Methods of Expressing Hydrogen Ion Concentration.—Figures, as used in the preceding paragraph to express ion concentrations, are obviously cumbersome. So extreme are the dilutions of H' and OH' ions dealt with in physiology, that all the fractions expressing their normalities are bound to have large denominators. A shorter way of writing them uses the negative powers of 10 to express the denominators. For exam-

¹ In 1908, L. J. Henderson began publication of data on hydrogen ion concentrations in blood etc., while in 1909 appeared Sørensen's memorable work on hydrogen ion concentration in relation to enzyme action together with methods of determination which have been the basis of the recent extensive work on the physiological significance of H' and OH' ions.

ple, C_{H^+} (concentration or normality of H^+ ions) = 2×10^{-6} means that the solution is $\frac{2N}{1,000,000}$. C_{H^+} is sometimes written as (H^+) , the brackets being used conventionally to indicate concentrations. If it is desired to avoid a coefficient the concentration may be expressed as a fractional power of ten. For example, $2 \times 10^{-6} = 10^{-5.7}$, since 2 is, by common logarithms, $10^{0.30}$. The advantage of the first expression is clear. One may see at a glance that 2×10^{-6} is twice as much as 10^{-6} or that 0.5×10^{-7} is one half of 10^{-7} . It is not so readily apparent that $10^{-5.7}$ is twice 10^{-6} or that $10^{-7.3}$ is one half of 10^{-7} . One has to become accustomed to thinking in negative logarithms. There is, however, a very good reason for using them. In computations of C_{H^+} and C_{OH^-} , based upon the methods of measurement to be described presently, the result is a common logarithm. Sørensen (1909), in his classic work establishing the modern methods of acidity determination in biological work, suggested that if the logarithm itself were used the labor of translation into antilogarithms would be avoided. The concentrations of H^+ and OH^- met with in all biochemical measurements are fractions of normal so that only negative logarithms appear. Sørensen proposed to drop the negative sign and merely express the concentration by a positive number numerically equal to the negative logarithm. He used the abbreviation pH to stand for the power (logarithm) of the number expressing the concentration of hydrogen ions. In his notation, then, $C_{H^+} = 2 \times 10^{-6}$ becomes $pH = 5.7$. Since the negative sign is omitted, *the pH of a solution really represents the logarithm of the reciprocal of the concentration of hydrogen ions.* Sørensen's notation has been universally adopted. Since every chemical or physico-chemical change in living matter, in other words every physiological activity, is markedly influenced by any change in concentration of hydrogen ions, C_{H^+} has to be taken into account in the majority of biological investigations. As a result, one can scarcely read any recent physiological literature that does not employ the expression pH.² Although it is at first confusing to represent increasing acidities by numerically decreasing numbers, the slight effort involved in becoming accustomed to the convention is worth while.

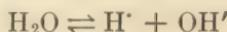
Methods of Expressing Values of Alkalinity.—The concentration of hydrogen ions may be used to express alkalinites as well as acidities; because, for any given C_{H^+} , a certain definite corresponding C_{OH^-} must prevail in any watery solution. It is apparent that conversely C_{OH^-} denotes a certain definite C_{H^+} but the concentration of hydrogen ions is commonly given to express acidity, neutrality or alkalinity, for the

¹ If the student will recall that the algebraic notations 10^2 , 10^1 , 10^0 , 10^{-1} , 10^{-2} , etc. are equivalent to 100, 10, 1, $\frac{1}{10}$, $\frac{1}{100}$ etc., the use of negative exponents becomes clear.

² The form pH is most generally accepted but is sometimes written P_H or p_H .

simple reason that our methods for its precise determination are well developed while those for obtaining $C_{OH'}$ are not equally convenient.

The explanation of the constant correspondence between them is found in the law of mass action. The reaction:



is a reversible one and therefore tends to attain an equilibrium. Like all such equilibria it is determined by the relative masses (concentrations) of the substances involved in the reaction. This simply means that if the relative abundance of water is increased the reaction is driven from left to right, while if either H^+ or OH' or both become relatively more abundant, the reverse reaction occurs. The law expressing this equilibrium may be epitomized in the following formula:

$$KC_{H_2O} = C_{H^+} \times C_{OH'}$$

in which K represents a constant, C_{H_2O} the concentration of water molecules (gram molecules of water per liter of solution) and C_{H^+} and $C_{OH'}$ have the usual significance. Written in the following way:

$$\frac{C_{H^+} \times C_{OH'}}{C_{H_2O}} = K,$$

the formula will perhaps make more apparent the fact that as C_{H^+} increases, C_{H_2O} remaining unchanged, $C_{OH'}$ must correspondingly decrease. By suitable measurements this relation has been proved to hold true in various watery solutions, containing comparatively low concentrations of H^+ and OH' ions.

The law of mass action, of which this is one application, does not hold true for concentrated solutions of strong electrolytes, except one makes certain modifying assumptions. This fact need not interfere with the use of the above formula for the explanation of H^+ and OH' ion concentrations as encountered in biochemical work. Concentrated solutions of strong acids and strong bases, such as N HCl or N NaOH do not have to be considered in connection with measurements of C_{H^+} of blood or any other biological fluids. All of the latter are so low in concentration of H^+ and OH' ions that measurements upon them are in satisfactory agreement with the equation as here represented. The student should not gain the idea that the law of mass action is invalidated because it has limitations. It is, in common with all human knowledge, still incomplete.

In this formula, K, called the equilibrium constant, does not represent a static equilibrium but a dynamic one. K merely indicates that the rate (velocity) of dissociation into H^+ and OH' ions bears such a relation to the rate of association of these ions into H_2O molecules that K is maintained a constant.

Returning to the point at issue—the expression of alkalinity in terms of C_{H^+} —it is clear that if we know K for the reaction $H_2O \rightleftharpoons H^+ + OH'$

and C_{H_2O} and can determine C_H , we may at once compute $C_{OH'}$. As a matter of fact, the information required is somewhat simpler. In all determinations of acidity or alkalinity, the actual values for C_H and $C_{OH'}$ are very small in proportion to C_{H_2O} . This is particularly true for the very mildly acid or alkaline reactions met with in biological fluids. Consequently the change produced in C_{H_2O} , when a certain number of molecules of water are produced by neutralization, is so small as not to affect any computation based on measurements of even the highest accuracy attainable. The whole expression $K \cdot C_{H_2O}$ may therefore be regarded as a constant. Its value has been determined from measurements of C_H and $C_{OH'}$. At about 22°C., the value of this constant is 10^{-14} . It varies slightly with change of temperature; but as results obtained at any temperature may be reduced, if great accuracy is required to those which would be obtained at 22°C. and as the exponent, -14, is more easily remembered than a fractional one, 10^{-14} is the value commonly employed. Since it is really $K \cdot C_{H_2O}$ it is often represented as K_w ; so that $K_w = C_H \times C_{OH'}$ and $\log K_w = \log C_H + \log C_{OH'}$ or $14 = pH + pOH$. If, then, a solution has $pH = 6.7$ ($C_H = 10^{-6.7}$), its $pOH = 7.3$ ($C_{OH'} = 10^{-7.3}$).

The following deductions are drawn from these formulas: (1) Every neutral solution, since it has equal concentrations of hydrogen and hydroxyl ions, must have $pH = 7$; (2) every acid solution has a pH numerically less than 7 and the smaller its pH the greater its acidity; (3) every alkaline solution has its pH numerically more than 7 and the larger its pH the greater its alkalinity; (4) a solution, normal in hydrogen ions has $pH = 0$; and (5) a solution normal in hydroxyl ions has $pH = 14$. If one carries in mind an imaginary scale, such as is represented below, all pH values soon come to have a very clear and definite meaning.

$C_{OH'}$	C_H	pH	
10^0	10^{-14}	14	Represents pH value of a solution normal in hydroxyl ions.
10^{-1}	10^{-13}	13	
10^{-2}	10^{-12}	12	
10^{-3}	10^{-11}	11	
10^{-4}	10^{-10}	10	
10^{-5}	10^{-9}	9	
10^{-6}	10^{-8}	8	
10^{-7}	10^{-7}	7	Represents pH value of a neutral solution.
10^{-8}	10^{-6}	6	
10^{-9}	10^{-5}	5	
10^{-10}	10^{-4}	4	
10^{-11}	10^{-3}	3	
10^{-12}	10^{-2}	2	
10^{-13}	10^{-1}	1	
10^{-14}	10^0	0	Represents pH value of a solution normal in hydrogen ions.

↑
Increasing alkalinity
↓
Increasing acidity

Log K_w , in the equation $\log K_w = \log C_H + \log C_{OH'}$, is taken as equal to -14 , its value at approximately 22°C .

This scheme does not represent the fractional values of pH; but if one remembers that a pH of 5 represents an acidity 10 times as great as that stated to be pH = 6, it is not difficult to imagine each space between unit intervals as subdivided in logarithmic ratio,—as though a small slide-rule were laid down upon it. Figure 32 shows such a scale, the one on the left. Comparison of the two scales will suggest computations that give familiarity with the use of negative logarithms. The pH values

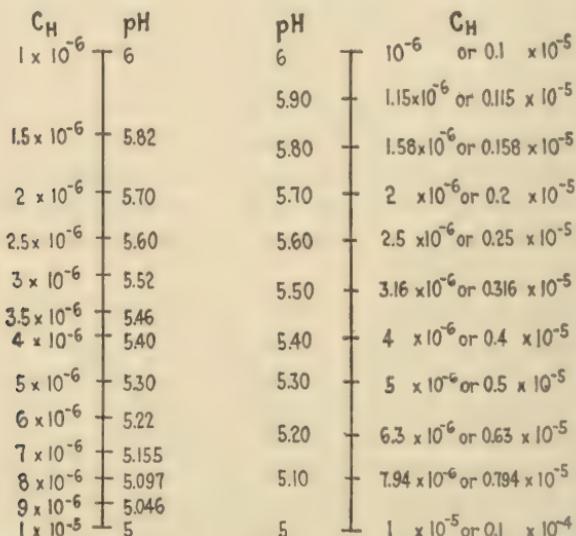


FIG. 32.—Scales for comparison of the fractional values of pH. The scale on the left compares regular changes in C_H values with the corresponding pH values; the one on the right compares regular changes in pH with the corresponding C_H values. The scale on the left is laid off in logarithmic spacing as upon a slide rule. A study of these scales helps to clarify the puzzling relationships between negative logarithms. For example, a solution of pH = 5.7 is twice as acid as one of pH = 6. A solution of pH = 5.3 is five times as acid as one of pH = 6 and half as strongly acid as one of pH = 5.

are given to only two or three places of decimals. This is the usual custom, as further accuracy is seldom justified by the exactness of measurements.

The Indicator Method for pH Determination.—Any method for determination of pH must be a static one, that is, it must not disturb the conditions in the solution that affect dissociation of H⁺ ions. As explained above, titration does cause further dissociation and therefore cannot be used for this purpose. Of the several different procedures used, the indicator method is the simplest, most convenient and most frequently employed. In principle, the method consists in addition to

the solution to be tested of a small quantity of a suitable indicator and comparison of the resulting color with those of the same indicator in a series of standard solutions of known pH. The unknown is taken to have the pH of the standard that it matches in color. Different indicators change color at different hydrogen ion concentrations. This is clearly illustrated by the results of titrations of the same solution against the same standard but with different indicators. For example, 10 cc.

of human gastric juice might require 10 cc. of $\frac{N}{10}$ NaOH to enable the indicator, phenol phthalein, to change from colorless (acid condition) to pink (alkaline condition); but the same quantity of juice might require only 2 cc. of the same alkali to turn the indicator, methyl orange, from pink (acid) to yellow (alkaline). This difference is due to the fact that phenol phthalein changes color in a range of pH values between 8.3 and 10, while methyl orange changes in the range between 3.1 and 4.4. It is evident that phenol phthalein while in the colorless, supposedly acid, condition may be nevertheless in an alkaline solution and that methyl orange after it has turned to the yellow, supposedly alkaline, color may still be in a solution whose true reaction is decidedly acid.

Theory of Indicators.—Indicators, in general, are organic dyes that are either weak acids or weak bases, so that they tend to dissociate to a very mild degree. When in the dissociated condition they furnish ions which impart to the solution a color distinctly different from that given by their undissociated molecules. Most of them are compounds which, at the time they change from dissociated to undissociated condition or vice versa, undergo an additional alteration in the form of some internal molecular rearrangement, called a tautomeric change. It is the combination of dissociation with tautomeric change which accounts for the fact that indicators do not suddenly shift from one color to another as the pH of the solution gradually changes during titration with very dilute standard solutions. The essential cause of the color change is, however, electrolytic dissociation. That is the reason why indicators may be used in hydrogen ion determination. How shall we explain the fact that a given indicator shows its dissociative color change within a certain range of hydrogen ion concentration? Stripped of its mathematical theory it amounts, for practical purposes, to this: The indicator has only a limited power of dissociation, so that if it is itself an acid, there will be a certain pH at which hydrogen ions will be so abundant in the solution that it can no longer avoid combining with them to form undissociated molecules. Similarly, if the indicator is itself a base, there will be some concentration of hydroxyl ions (corresponding, of course, to a certain pH) which prevents dissociation. The details of the theory of indicators cannot be given space for treatment here. The reader is referred to Clark's excellent summary for this and other discussions of

the theory and practice of hydrogen ion determination. His extended bibliography will also be found very useful.

That indicators do actually show changes in hydrogen ion concentration rather than some other chemical change is more vividly apparent if one notes the colors of the same indicator in different concentrations of the same acid. Methyl violet (crystal violet), in hydrochloric acid of concentrations; 2N, N, $\frac{N}{10}$, $\frac{N}{100}$, $\frac{N}{1,000}$, gives respectively the colors:

Golden yellow, green, greenish blue, blue and violet. The same concentration of dye is added in each case and the only difference between the various acid solutions is in the proportion of water added.

Choice of Indicators.—By a suitable choice of indicators, color changes may be obtained at any range of pH values. Many different indicators have been used in the course of development of methods. Most of these indicators have been found unsuitable for general biological work. Certain criteria are to be fulfilled: (1) The indicator should be a definite compound¹ that may be prepared in a standardized state of purity; (2) it should show very brilliant color in great dilution so that the concentration of indicator, required to produce satisfactory color in a test or standard solution, will not be sufficient to appreciably disturb the balance of H' and OH' ion concentrations; (3) it should give no disturbing reactions with constituents of the particular solution to be tested, so that various indicators which combine with proteins or with salts are not useful for most biological work; and (4) it must assume a definite color very quickly after addition to the solution and hold the same color as long as the pH of the solution remains unchanged.

After trial of many synthetic dyes and consideration of many indicators used by other investigators, Clark and Lubs have selected the series of indicators that they believe most nearly conform to requirements for biological work. The eight dyes in the series are so selected that their several color changes provide for the detection of the entire range of pH values ordinarily encountered in biological fluids and reagents. The following table, taken from Clark, shows the series "with the common names which they (Clark and Lubs) suggested for laboratory parlance, the concentration of indicator convenient for use, a rough indication of the nature of the color, and the useful pH range."

These indicators have been so widely used with satisfactory results that they bid fair to replace the use of the older ones. Most of them belong to the sulphone phthalein series. Thymol blue occurs twice in the list, because it has two useful ranges of color change.

¹ This is one reason why natural pigments such as litmus, alizarine, red cabbage pigment (all of plant origin) and cochineal (of animal origin) are discarded in favor of the synthetic dyes.

Chemical name	Common name	Concentration per cent	Color change	Range
Thymol sulphon phthalein (acid range)	Thymol blue (see below)	0.04	Red-yellow	1.2-2.8
Tetra bromo phenol sulphon phthalein	Brom phenol blue	0.04	Yellow-blue	3.0-4.6
Ortho carboxy benzine azo di methyl aniline	Methyl red	0.02	Red-yellow	4.4-6.0
Di bromo ortho cresol sulphon phthalein	Brom cresol purple	0.04	Yellow-purple	5.2-6.8
Di bromo thymol sulphon phthalein.	Brom thymol blue	0.04	Yellow-blue	6.0-7.6
Phenol sulphon phthalein	Phenol red	0.02	Yellow-red	6.8-8.4
Ortho cresol sulphon phthalein	Cresol red	0.02	Yellow-red	7.2-8.8
Thymol sulphon phthalein	Thymol blue	0.04	Yellow-blue	8.0-9.6
Ortho cresol phthalein	Cresol phthalein	0.02	Colorless-red	8.2-9.8

Procedure in Colorimetric Determinations.—The first procedure for determination of pH in a solution is to test small portions of it with one or two indicators to get an approximation. Having thus found its general reaction one chooses an indicator likely to cover the proper range and adds 3 or 4 drops of its solution to 10 cc. of the solution to be tested. The color, thus developed, is compared with the colors of a series of standard solutions, similarly prepared with the same indicator, and varying in pH by small known intervals but all within the range of the useful color changes of the indicator. The standard solutions are prepared in accordance with methods to be described presently (p. 168). The final basis of their standardization is the electrometric method (p. 170). Having found a pH it is best to check the result by means of similar procedures, using in turn each of the other indicators which show useful color changes within the same region of pH values. It is important to employ test tubes of the same bore and of uniform thickness and color of glass. The smaller the intervals between the pH values of the several standards used, the closer may one estimate the result; but greater care in matching of colors is required. Although mere inspection will do for rough work, a color comparator enables one to obtain finer results. Uniformity of lighting is obviously an advantage so that high accuracy demands the use of artificial light. When only a small amount of solution is available for the test, drops of it, placed near similar drops of standard solutions upon a white plate, may be used for color comparison after

adding the appropriate indicator. Clark and Lubs recommend that the standard solutions differ in pH by intervals of 0.2. By interpolation, one is then able to estimate pH values to within 0.1. This is often a very satisfactory degree of precision. For rough estimations and for illustration purposes, the chart of indicator color changes, given by Clark, will be found useful.

Standard Buffer Solutions.—It is apparent that the difficult and laborious and yet most important part of colorimetric estimations of pH is preparation of the standard solutions. Strong acids or bases in pure water are utterly unreliable since their pH values would change in accordance with absorption of CO₂ from the air and because of other disturbing factors. Substances which tend automatically to stabilize the hydrogen ion concentration of their solutions are needed.

The nature of the self-regulating mechanism which checks changes in pH as effectively as a governor automatically regulates the speed of an engine, is worthy of careful study. Besides explaining the preparation of standard solutions, it gives the fundamental explanation of the regulation of hydrogen ion concentration in living things and that, we have already shown, is a matter of life and death. Incidentally, an understanding of the mechanism, about to be described, has been indispensable in the development of that part of modern medicine concerned with acidosis—the disturbance of the self-regulating mechanism of pH control—in the human body.

To show how stability may be obtained, the use of phosphates for standard solutions as employed by Sørensen and developed by Prideaux will be described. There are three kinds of phosphate salts, the alkaline, such as K₃PO₄, the dibasic, K₂HPO₄, and the acid phosphates, as KH₂PO₄. The first two are alkaline because they produce hydrolytic dissociation of water. K₂HPO₄ tends to dissociate thus:



and simultaneously water dissociates:



but the dissociative power of phosphoric acid is not sufficiently great to retain a concentration of PO₄^{'''} ions corresponding to the concentration of H⁻ ions thus arising from the dissociation of water. Consequently, some of it is present, no doubt, as H₃PO₄ molecules and some as HPO₄^{''} and H₂PO₄['] ions; since PO₄^{'''} combines with H⁻ ions. This combination leaves a preponderance of OH⁻ and K⁺ ions, which are able to remain in the dissociated state. Although there are other possibilities, the chief final result of the process may be represented thus:



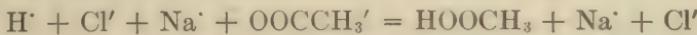
The acid phosphate dissociates chiefly in this manner:



If K_2HPO_4 with its preponderance of OH^- ions and KH_2PO_4 with a similar excess of H^+ ions be mixed in the right proportions, the result is a neutral solution. But it is a solution of peculiar properties; for if now we add acid (H^+ ions) to it, there is a shift of HPO_4^{2-} to H_2PO_4^- ; so that many of the H^+ ions, that are added, disappear and their net increase is very small. Similarly, upon the addition of alkali (OH^- ions) a shift of H_2PO_4^- to $\text{HPO}_4^{2-} + \text{H}^+$ ions occurs. Many of the H^+ ions so produced combine with the OH^- ions so that again the net change is very small. When enough acid has been added to use up all the HPO_4^{2-} ions or when enough alkali to use up all the H_2PO_4^- ions, additions of further amounts will cause more rapid changes in pH values. A mixture of K_2HPO_4 and KH_2PO_4 will, however, in the range between the values pH 5.8 and pH 8 show a remarkable power to "absorb," as it were, a large proportion of either H^+ or OH^- ions added to it. A solution showing this property is called a buffer solution from a fancied analogy between its action and that of shock-absorbing "buffers" on machines.

There are many combinations which act in a similar way to produce the buffer effect in solutions. As we shall see in our discussion of the pH of the blood, carbonates and bicarbonates give, because of the extremely low dissociation constant of carbonic acid, the most efficient buffer combination known. They are not practical to use in standard solutions, however, because they would give up CO_2 whenever exposed to the air and would therefore gradually change in pH values. The combination of phthalates and acid phthalates has been developed with great success to give standard buffer solutions of pH values ranging from 2.2 to 6.2. The basic and acid borates are valuable for buffers in the alkaline range from pH 7.8 to pH 10. The citrates have also been used. In short the salts of all very weakly dissociating di- and tri-basic acids are especially effective.

Other types of buffers exist. A strong acid such as HCl with a salt of a weak acid and a strong base, such as sodium acetate (NaOOCCH_3), produce the effect. Although sodium acetate dissociates freely, acetic acid is only slightly dissociated; so that part, at least, of the reaction between hydrochloric acid and sodium acetate may be represented thus:



In other words, acetates and similar salts cause the disappearance of some of the hydrogen ions which are added to their solutions. For strong acids, such as $\frac{N}{10}$ HCl , a strongly dissociating salt with the same anion (e.g. KCl) gives a buffer effect; since, if present in large amounts, further dissociation of the acid is checked by the high concentration of the anion.

Any weakly dissociating amphoteric electrolyte is a buffer, since it can absorb either H' or OH' ions as they tend to increase in the solution. Because of this fact amino-acids have been employed as buffers in standard solutions. Except for the expense of purification and other practical difficulties, both amino-acids and proteins might be used as buffers. They are very effective in this respect.

In actual practice standard buffer solutions are made up by mixing varying proportions of a standard acid or alkali solution with a fixed quantity of a solution containing the buffer substance in suitable concentration. The solutions from which standards are made require care and skill in preparation if they are to have the correct hydrogen ion concentrations. Since upon their accuracy depends the precision of the entire process, biological and commercial laboratories, not suitably equipped for careful quantitative chemistry, will doubtless do well to obtain the solutions from a reliable chemist. The rapid increase in the use of indicators and standard buffer solutions has made their purchase easy.

The Electrometric Method for Hydrogen Ion Determination.— At this point the reader is doubtless asking: How are pH values of any standard solutions actually determined, so as to give an initial starting point to any system of comparative measurement? The hydrogen ion concentration of a pure acid dissolved in pure water may be reckoned from the osmotic pressure (determined by lowering of the freezing point) or from the electrical conductivity of the solution. In buffer solutions, however, many varieties of ions are present and such methods cannot be applied. The problem has been solved by the use of the hydrogen electrode in the electrometric determination of hydrogen ion concentration. The physico-chemical principle involved in this method will now be taken up even though details necessary for its practical use are not profitably presented in a work of this character. The reader is referred to current works on physical chemistry for a more elaborate treatment.¹

The Theory of Electrolytic Solution Pressure.— Since an ion is electrically charged, any method which singles out, for measurement, the charges upon one kind of ions measures their abundance in a given solution. Any one ion carries a definite number of unit charges; so that the intensity (potential) of the charge borne by a given kind of ions can give the data for computing their concentration. In effect, the *hydrogen electrode* singles out hydrogen ions in such a way as to make their charges measurable. The underlying chemical principle is of a very general application, and is perhaps best understood in the light of the theory of the nature of solution. Any substance has, theoretically, a solubility in water; for even though it appears to be quite insoluble, there is some attractive force (electromagnetic) between its molecules and those of

¹ Clark, "Determination of Hydrogen Ions," gives a full account.

water which, at the interface between them, produces some ions of the substance. They bear the charges characteristic of their species.

Since the charge is capable of quantitative determination it is taken as a measure of the attractive force between the substance and water. This quantity is known as the electrolytic solution pressure or solution tension of the substance and is a characteristic number for each of the chemical elements. When a charge is called out upon the surface of any body an equal charge of the opposite sign is bound to be left in the body itself. The effect may be illustrated by the behavior of metallic copper in water. The copper tends to send out copper ions bearing positive charges. For this reason the body of copper itself becomes negatively charged. The two charges, being equal and opposite, hold close to each other so that they remain constant and no copper ions can actually go off into solution in the water unless a compensatory charge be supplied by some other force (acid or salt action, hydrolytic dissociation of water, the electric current, etc.). Pure metallic copper only dissolves to a very small extent in pure water as the process soon reaches an equilibrium. Just as certain forces can drag Cu⁺ ions, with their attendant positive charges, out into the solution, so other forces can drive them back into the metallic copper. Copper ions added to the solution can do just that. The adjustments between copper and water at their contact face are much the same as those between a liquid and a gas. At the surface between water and air, water molecules go off by evaporation until the gas pressure of water vapor just balances the vapor pressure of liquid water. So at the boundary between copper and water, copper ions diffuse into the water until osmotic pressure of copper ions in solution just balances the electrolytic solution pressure of metallic copper. As soon as this equilibrium is reached, the charge upon the copper and the concentration of copper ions in solution remain constant unless some force disturbs the equilibrium. Suppose the concentration of copper ions to be increased by adding copper sulphate to the water. The result may be foreseen if one again thinks of the water-air analogy. If water vapor becomes sufficiently concentrated in the air, rain falls. In terms of physics, pressure of water vapor becomes greater than the vapor pressure of water; but the crux of what we are explaining lies in the fact that it is the gaseous molecules of water alone which cause condensation. The pressure due to other gases is not concerned. In an analogous way, it is the pressure of copper ions in a solution, and those alone, which determines the magnitude of the electric charge carried by pure copper immersed in the solution. In the case of copper ions, indeed, the charge upon metallic copper may even be reversed to a positive one since the pressure of copper ions may drive so hard against the solution tension of the metal that they actually confer their charge upon it. To one unaccustomed to electro-chemistry the analogy between gas pressure and ion pressure may seem far fetched; but the same funda-

mental properties of matter underlie both processes and the two mathematical formulas expressing respectively, the quantitative aspects of each process have the same form. (See p. 178.)

The Concentration Cell.—It may occur to the reader that we are dealing with the principles which explain the action of galvanic cells. Such is the case. The simplest of galvanic cells, the concentration cell, may well be used to explain the application of electrolytic solution pressure to the problem in hand. Suppose two pieces of copper are immersed in a dilute solution of copper sulphate so that one of them is near the surface and the other near the bottom of the containing jar where a mass of copper sulphate crystals is placed. (See Fig. 33.)

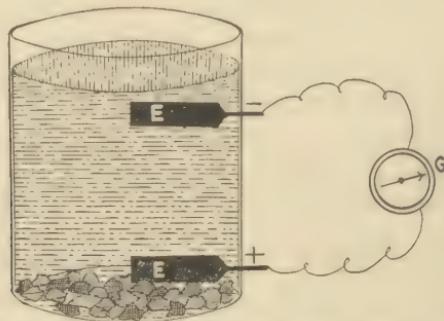


FIG. 33.—A simple copper concentration cell. The jar contains saturated copper sulphate solution at the bottom and dilute copper sulphate solution above. E, E , represent electrodes of pure copper; G , a galvanometer. Further description in the text.

Copper sulphate dissolving from the crystals will make the solution at the bottom of the jar nearly or quite saturated and gravity will keep the resulting heavy solution, if stirring be avoided, at the bottom. Diffusion of copper sulphate through the solution will be so slow that for a time there will be a marked difference between the concentrations of copper ions in the upper and lower layers. As long as the difference holds there will be a different charge upon the two strips of copper so that if they are connected by suitable conducting wires through a galvanometer the difference in their potential (charge) creates an electromotive force which causes a flow of current through the galvanometer. The amount of its deflection may be used to measure the difference of potential (voltage) between the two pieces of copper which may now be called electrodes since they are the terminals of the metallic part of the electrical circuit. Both electrodes are electrically charged but at different potentials. The greater concentration of copper ions in the saturated solution has opposed the solution pressure of its electrode to a greater extent than has the smaller concentration in the dilute solution

against the other electrode. Provided the electrodes are of pure copper, only concentration of copper ions can affect their respective potentials. As the charge upon one ion is a well established quantity the charge due to any given concentration of ions can be reckoned. The difference in the ion concentration of the two regions of the cell bears a direct ratio to the difference in electrode potential. The voltage between the two electrodes, then, has only to be divided by a well established constant factor to compute the difference between the two concentrations of copper ions. The actual concentration of copper ions in a given pure copper sulphate solution may be obtained by measurement of its osmotic pressure or by other means such as conductivity measurements. It is evident, then, that, knowing the concentration of copper ions in one solution and the difference in potential between a pure copper electrode in it and a similar one in any complex solution containing copper salts, the concentration of copper ions in the second solution may be computed. The principle of the concentration cell has been applied to mercury acting against mercury salts in solution and to many other similar combinations. In actual practice the gravity form is less useful than a more permanent construction which connects two solutions of different concentration by porous clay or some other device to check free diffusion while still maintaining a liquid connection between them.

The Hydrogen Electrode.—If the foregoing discussion or, better still, actual use of concentration batteries, can make clear the relation between electrode potential and ion concentration, the principle of the hydrogen electrode is easily understood. Just as copper may be opposed to copper ions or mercury to mercury ions, so pure hydrogen gas may be allowed to exert its electrolytic solution pressure against the osmotic pressure or, what amounts to the same thing, the concentration of hydrogen ions. The technical difficulty is to find a way to use hydrogen gas as an electrode. This has been possible to do because colloidal platinum or palladium holds hydrogen very firmly adsorbed upon the surfaces of its minute particles. It is only necessary, then, to deposit colloidal platinum or palladium upon a metallic electrode and keep it in the presence of hydrogen to have a layer of the gas so completely surrounding the electrode that only hydrogen can be in contact with a surrounding solution. Strips of gold or platinum are electroplated in a solution of platinic chloride or palladium chloride so as to give them a complete coat of black, "spongy" metal. The hydrogen used to saturate them must be highly purified. It is either allowed to flow over the electrode as in the forms typified by the Hildebrand and the Bunker electrodes or is allowed completely to displace all air from an air-tight chamber, containing the solution to be tested and the electrode, as in the forms of which the Clark model is a well known example.

The Hydrogen Ion Concentration Cell.—Two hydrogen electrodes, each of which is immersed in a solution, may be connected by suitable conducting wires, leading through a voltameter or other electrometric device, while the circuit is completed by a liquid junction between the two solutions. A pair of hydrogen electrodes is represented diagrammatically in Fig. 34, in which H_2 represents the stream of pure hydrogen gas against both electrodes, C and C' indicate two solutions of differing concentration of hydrogen ions, L represents a liquid junction between

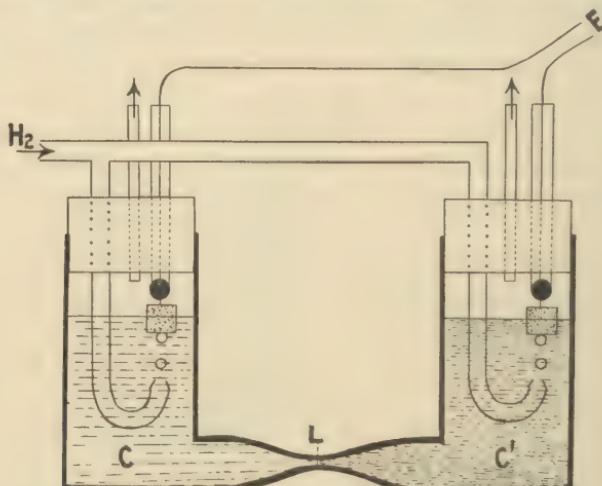


FIG. 34.—A gas chain or concentration cell, employing hydrogen electrodes. Explanation in text. (After Clark, "The Determination of Hydrogen Ions," Williams and Wilkins).

them and E represents wires which complete an electrical circuit by connecting both electrodes to an electrometric device. Because the two solutions possess differing C_{H^+} values the arrangement constitutes a concentration cell. It will yield a measurable voltage which can be determined and is due solely to the difference between the hydrogen ion concentrations of the two solutions. If the pH of one is known, that of the other can be computed from the observed voltage. Such an arrangement, since it uses electrodes covered with gas, is known as a gas chain.

The Calomel Electrode.—The use of two hydrogen electrodes, as diagrammed, served as the starting point of fundamental investigations in hydrogen ion determinations but in practical work such an arrangement has serious limitations. Obviously a solution of pure acid, of a hydrogen ion concentration that had been accurately determined in conductivity or osmotic measurements, would have to be used in one half of the cell. The difficulties involved in the standardization of such a solution can only be appreciated by the experienced physical chemist. To preserve its constancy of pH value in the absence of a buffer is also difficult. There is no practical way to determine the potential upon one electrode (*i.e.*, a

half cell) without completing an electrical circuit through a liquid juncture and another electrode. It is a great advantage, therefore, to be able to substitute for one half of the hydrogen ion concentration cell, a solution and electrode which so react upon each other as to furnish a known and dependably constant potential. The most convenient combination and the one ordinarily used is a potassium chloride solution and a so called calomel electrode. The entire arrangement including the solution, is often included in the term calomel electrode. The potassium chloride must be of the highest purity obtainable and its solution of accurately known strength. Either 0.1 N, normal or saturated solution may be chosen. The electrode must also be composed of highly purified materials: Metallic mercury and mercurous chloride (calomel). A discussion of the reasons for the selection of mercury, calomel and potassium chloride to form the half cell would lead us too far afield from our subject. The reader is referred to standard works of physical chemistry. The choice of potassium chloride deserves brief notice since it, better than any other known substance, avoids the production of disturbing potentials at the point where its solution makes the necessary contact with the one under measurement. The arrangement which constitutes the complete circuit, then, is according to the following scheme:

↙(platinum electrode)—(hydrogen)—(solution to be measured)
(volt measuring device)

↘(mercury)—(calomel)—(potassium chloride solution)↗

Calculation of pH Values from Observed Electro Motive Force.—If a voltmeter is used for direct reading of the electromotive force of the chain a certain rapidity and simplicity of manipulation is attained but at great expense of accuracy. Voltmeters are only constructed so as to give readings accurate to within about ten millivolts (a millivolt is 0.001 volt) while some hydrogen electrode work justifies readings in hundredths of a millivolt. Moreover the voltmeter permits current to flow between the electrodes and thus reduces the e.m.f. even while the determination is being made. For accurate work voltage is obtained by some modification of the potentiometer¹ principle. A standard equipment for hydrogen electrode work is shown in Fig. 35.

Computation of pH from the observed e.m.f. depends on the use of certain constants. One of them is the difference in potential corresponding to a given change in hydrogen ion concentration. This has been determined in various series of measurements. If, for example, a calomel electrode is connected, at a temperature of 18°C., to a hydrogen electrode in a solution known to have $C_H = 10^{-5}$ (pH = 5) and then the same electrodes are again connected, but this time the hydrogen

¹ The potentiometer and its use may be studied in any standard work on physics. The writer has found the catalogue of Leeds and Northrup especially helpful for discussion of the construction, theory and use of potentiometers.

electrode is in a solution known to have $C_H \cdot 10^{-6}$ ($\text{pH} = 6$) there would be shown in the second case an e.m.f. larger by 0.05772 volt than that found in the first measurement. Sufficient numbers of measurements have established the relationship that, for every change of one whole unit in pH value, the potential of the hydrogen electrode changes at 18°C . by 0.05772 volt. This value varies with temperature in such a manner that it is always equal to $0.00019837 T$, T being the absolute temperature. One other standard value is required: The difference of potential between

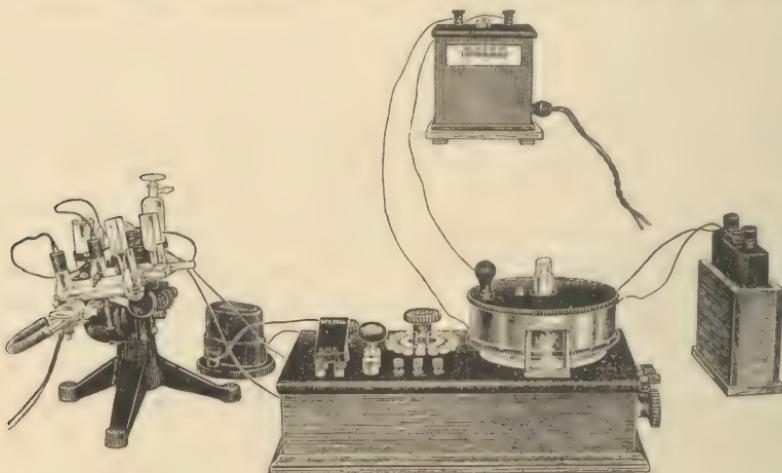


FIG. 35.—A standard equipment for electrometric measurement of hydrogen ion concentration. At the left, mounted on a tripod, is the Clark form of rocking hydrogen electrode with tube connections for the inflow of hydrogen gas. This electrode is rocked by a small electric motor attached to the tripod. The calomel electrode and the apparatus for making its liquid junction with the hydrogen electrode are also mounted on the tripod. Near the tripod is a standard Weston cell for standardizing the potentiometer. The latter is in the foreground. To the right of it is a storage battery for supplying the potentiometer current against which the potential of the hydrogen electrode is measured. Above is a shelf form of galvanometer provided with an internal electric lighting device. The hydrogen and calomel electrodes, the Weston cell, the storage battery and the galvanometer are all connected to the potentiometer through binding posts on its rear wall. (From the catalogue of The Leeds and Northrup Co.)

the calomel electrode and the hydrogen electrode when the latter is in a solution normal in hydrogen ions. This value can be inferred from measurements on a series of acids of known pH and is equal to 0.3380 volt at 18°C ., when the calomel electrode is in 0.1 N KC1, the solution commonly used for this standardization. Inasmuch as a solution, normal in hydrogen ions, has $\text{pH} = 0$, the e.m.f. (voltage) measured with an unknown solution around the hydrogen electrode at 18°C ., is equal to $0.3380 + 0.05772 \times (\text{pH of the unknown solution})$ or, if E represents the observed voltage, $E - 0.3380 = 0.05772 \times \text{pH}$ or

$$\text{pH} = \frac{E - 0.3380}{0.05772}$$

This relationship between the observed voltage and the pH is shown graphically in the following curve. (Fig. 36.)

Instead of the value, 0.3380, some other must be used if the temperature or the concentration of KCl upon the calomel electrode be changed. The relationship of observed voltage to pH, when a saturated solution of KCl is used with the calomel electrode, is shown in the curve. Inasmuch as all the standard values in the above equation are greatly affected by temperature changes, electrometric measurements must be made in a thermostat at constant temperature if great precision is expected. An

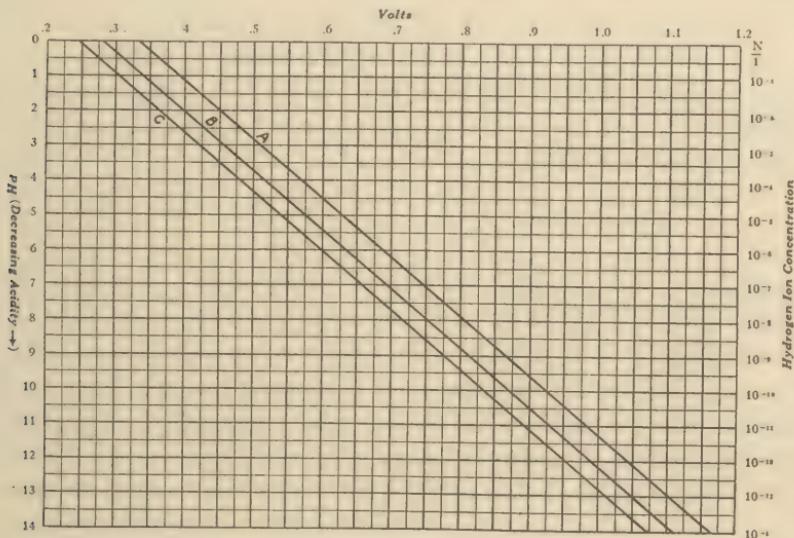


FIG. 36. Curves showing the relationship between hydrogen ion concentration and the observed voltage between a hydrogen electrode and a calomel electrode. A, when the calomel electrode contains a 0.1 N solution of KCl; B, when the calomel electrode contains a N solution of KCl; C, when the calomel electrode contains a saturated solution of KCl. (From the catalogue of The Leeds and Northrup Co.)

additional refinement, correction for barometric pressure when the hydrogen used is under atmospheric pressure, is not usually required for biological measurements. It is also important to appreciate the fact that any so-called "constants" in a quantitative expression of the relationships among natural phenomena are valid only so long as all the conditions upon which they rest are maintained constant. It may be mentioned in passing that the constants here employed are not entirely agreed upon with respect to the last places of decimals. Investigators are not able to produce an absolute constancy of surrounding conditions. It is nevertheless true that electrometric determination of hydrogen ion concentration is by far our most accurate method of acidimetry and alkalimetry.

The above equation is given as though it were entirely based on empirical measurements. This seems justified since the values used in it were

finally selected from just such data. As a matter of fact the fundamental formula is deduced from the application of the laws of gas pressure to the theory of the concentration cell. The deduction of the equation will be found in works on physical chemistry. Clark also gives a full treatment of this subject. Students who are willing to use calculus, which is for science "its most powerful and subtle instrument of research," will not fail to profit by mastering this subject. It involves some of the most fundamental relationships of physical science. It is sufficient to state here that the integrated equation, thus deduced, takes this form:

$$\log_{10} \frac{1}{C_{H^+}} = \frac{E - C \text{ (integration constant)}}{0.00019837 T}$$

When E represents the voltage between a 0.1 N calomel electrode and a suitably arranged hydrogen electrode and when T is 291° absolute (18°C.), this form becomes:

$$\log_{10} \frac{1}{C_{H^+}} = \frac{E - 0.3380}{0.05772}$$

The expression, $\log_{10} \frac{1}{C_{H^+}}$, is the logarithm of the reciprocal of the hydrogen ion concentration of the unknown solution. This, by definition, is the pH of the solution. The form of the integrated equation, then, is such that results computed by it are in pH units; so that Sørensen's convention for expression of hydrogen ion concentrations becomes a great convenience by removing the necessity for reducing the results to terms of C_{H^+} .

The accuracy of electrometric measurements so far exceeds that of other methods, that the hydrogen electrode should be used when precision is required in determination of C_{H^+} . With sufficient care, this method enables one to determine pH values to the third decimal place, whereas with the colorimetric method one can scarcely be sure of figures beyond the first decimal. Another advantage of the hydrogen electrode method is that turbidity, color or formation of precipitates, which might interfere with the use of indicators, do not interfere with electrometric measurements. The colorimetric method, on the other hand, has a distinct advantage for use in determining the pH of blood, because the CO_2 and O_2 of the blood complicate the use of the hydrogen electrode.

Practical Applications of Hydrogen Ion Measurements.—Hydrogen and hydroxyl ions are present during the progress of nearly all chemical reactions. This might well be inferred from what was said about water as a solvent and as an electrolyte. It is not surprising, then, that determinations of pH values enter into all sorts of chemical work. In numerous and diverse manufacturing industries the power to control the true reaction of the medium in which a process goes on may make all the

difference between a uniformly successful result and that uncertainty which produces occasional and unaccountable failure. Of many instances, which might be cited, we will select one as a biochemically interesting example. In bread making, the grain proteins, which form the glutinous texture, that enables dough to hold yeast gases and therefore rise evenly, are greatly altered in viscosity by changes in hydrogen ion concentration. Perhaps the bakeshop is the last place where we would expect pH values to be determined, but they may be found useful even there.

In agriculture, they seem likely to be of considerable value in determination of soil acidities. Colorimetric apparatus can be carried into the field and estimations made with less expenditure of time and labor than is required for titration. The results also are more easily interpreted than are those obtained by older methods. The value of such determinations lies in the fact that different plants are adapted to grow in soils where the moisture is of different hydrogen ion concentration. Acidity has long been known to be detrimental if not fatal to many crops. Hence the practice of using lime to neutralize the soil when necessary.

The natural distribution of species of plants has been shown to be correlated to the pH of the soil. Similarly, aquatic life, both plant and animal, varies sufficiently in sensitiveness to hydrogen ion concentration to enable one to detect its relationship to distribution of species. The pH of the ocean itself remains remarkably constant because of the buffer action of its salts. In comparatively hand-locked bodies or in stagnant fresh water, fermentations, due to acid-forming micro-organisms, may produce distinct changes in pH values.

One of the most remarkable applications of hydrogen ion determination has been in Bacteriology. A surprising degree of sensitiveness of bacteria and other micro-organisms to variation in pH of the surrounding medium has been shown. This has aided greatly in identification and artificial culture of forms both pathogenic and commercially useful. Equally notable are the variations in power of acid production among different microscopic forms. This too has been useful information. A complete discussion of the significance of these and similar investigations belongs more appropriately to the study of Bacteriology.

The applications to general physiology have already been partially indicated. A few especially significant outgrowths of the use of this comparatively new method may well be mentioned here. The nature of enzyme action has been seen in a new light. For this reason, an improved understanding of the animal digestive process, of animal oxidations and other phases of metabolism has been obtained. Because of this type of work, ideas concerning the process of respiration have required considerable revision. In medical diagnosis, such as the determination of pH values of urine, gastric juice and blood, this method has given some

advantages not attainable by older methods. It has certainly thrown brighter light upon relationships as fundamentally important in medicine as in general physiology, relationships between the factors which regulate the hydrogen ion concentration in animal fluids and tissues. As this subject demands some extended explanation it will be taken up in the following section.

The Regulation of Neutrality in the Animal Organism.—The uniformity, which prevails in the pH of blood is truly amazing if one can only appreciate the circumstances under which it is maintained. The net result of every process occurring in living cells of animal bodies is increased acidity. The chief product of oxidations is carbon dioxide. Although it is a very weak acid, the enormous quantities of it produced represent a very distinct acidity. In addition, such acids as lactic, butyric, oxalic and a long list of others are formed while the anions, $\text{SO}_4^{''}$ and $\text{PO}_4^{''}$, resulting from oxidation of proteins, must be neutralized if they are not to produce increase of hydrogen ions. It is true that the animal body has several safety valves for the escape of excess of potentially acid substances. One of these is the lungs, through which CO_2 excretion is chiefly effected. The other important one is the kidneys. They are able to eliminate any excess of basic or of non-volatile acidic substances. As a result, the pH value of the urine tends to follow very faithfully any changes in the potentially acid- or alkali-producing constituents of the diet. The urine of animals on a high protein diet, such as carnivores consume, will tend to be high in its content of phosphates and sulphates. Correspondingly, the C_{H} of the urine is comparatively high. Carnivores always have a distinctly acid urine. Herbivorous animals, on the other hand, consume, especially in the form of green herbage, comparatively large amounts of potassium and other basic salts. The corresponding excretion of potassium, etc. (explained in the preceding chapter) results in high pH values (low C_{H}) in their urines. All herbivores have a characteristically alkaline urine. Even the most acid fruit juices are so high in their content of alkaline salts that, since their acids are oxidized in the body to carbon dioxide, which is eliminated via the lungs, the net result of eating sour fruits is a decreased acidity of the urine.

The urine, then, contains the overflow of either acidic or basic substances which must be eliminated if the blood is to maintain constancy of its reaction. An interesting analogy should be noticed. Just as the kidney, more than any other organ, enables the so called higher animals to maintain the required degree of constancy in the osmotic pressure of their body fluids, so a similar regulation of pH, even more important physiologically, is also effected by the kidney. In maintenance of the hydrogen ion adjustment, the kidneys are more noticeably aided by the respiratory elimination of carbon dioxide, than they are in the matter of osmotic regulation.

Mere elimination of the excess of acid or basic substances could only compensate for changes in C_{H^+} of the blood, after they had actually occurred. As a matter of fact, they do not actually occur in the normal healthy body, to an extent detectable, save by the most refined and delicate measurements. Even in the conditions caused by the profound breakdown of the usual regulative process (extreme cases of human acidosis), the actual pH of the blood only changes from an average of 7.35 to 7.00. This is the fatal stage of what is called *acidosis*; but even then, the blood has not become really acid. It has only been changed to the neutral condition. This is true, in spite of the fact that comparatively large quantities of non-volatile organic acids are produced during such a disturbance of metabolism. From what was said above concerning standard solutions, it is evident that the blood possesses remarkable "buffer" powers.

Of the buffer substances in the blood, the bicarbonate combinations have been shown by Henderson to be the most effective. Like the system of phosphate salts, described above, bicarbonates dissociate to produce distinctly different results under slightly varying conditions. Though we need not take every possibility into account, we may represent sodium bicarbonate, an abundant constituent of blood, as dissociated thus:



but this causes a simultaneous dissociation of water:



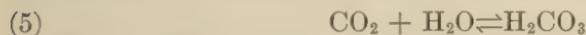
resulting in the formation of some carbonic acid molecules because carbonic acid has low dissociating power. Thus:



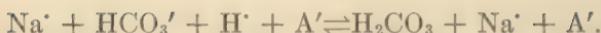
The net result of this is a slight excess of OH^- ions in the solution. It must be a very slight excess, however, because a tendency to increase in C_{OH^-} makes the reaction:



go from left to right. The equilibrium among these various reactions is such that it accounts chiefly for the slight normal alkalinity of blood. But consider some of the many forces tending to disturb this equilibrium. Carbon dioxide is constantly entering the blood from every active cell of the body. It tends to react with water thus:



It can scarcely dissociate at all since it must drive reaction (3) from right to left and in so doing disturbs the equilibrium of reaction (4). In case a stronger acid, such as lactic, enters the blood a similar compensation occurs. If we represent the acid by HA , A standing for any acidic anion, we may write the chief reaction resulting from acid addition thus:



This is due chiefly to the low dissociating power of carbonic acid. Since respiration removes CO_2 from the lungs the equilibrium of reaction (5) is disturbed as the blood circulates through the lung capillaries and any tendency to accumulation of carbon dioxide in the blood is counteracted. The efficiency of bicarbonates as buffers is strikingly illustrated in the following way, adapted from Henderson. Suppose 100 liters of 1 per cent NaHCO_3 solution be kept in equilibrium with an atmosphere containing one gram of carbon dioxide per liter. Suppose hydrochloric acid is added in successive small portions to the solution and that after each addition the solution is allowed to come to equilibrium with the atmosphere. The changes in hydrogen ion concentration of the solution, resulting from these additions of acid, are approximately represented in the following table:

HCl added, grams	Normality of hydrogen ions C_{H}^+	Normality of hydroxyl ions C_{OH}^-
0	0.57×10^{-7}	1.76×10^{-7}
10	0.59×10^{-7}	1.70×10^{-7}
50	0.68×10^{-7}	1.47×10^{-7}
100	0.83×10^{-7}	1.20×10^{-7}
150	1.08×10^{-7}	0.93×10^{-7} —approximate neutrality
200	1.54×10^{-7}	0.65×10^{-7}
250	2.60×10^{-7}	0.39×10^{-7}
300	10.00×10^{-7}	0.10×10^{-7}
310	22.0×10^{-7}	0.045×10^{-7}
318	260.0×10^{-7}	0.003×10^{-7}
320	450.0×10^{-7}	0.002×10^{-7}

This table shows that after enough acid has been added to break down nearly all of the NaHCO_3 and the solution has attained $C_{\text{H}}^+ = 2.6 \times 10^{-7}$ ($\text{pH} = 6.58$), there is a distinct loss of the buffer action so that subsequent additions of small amounts of acid produce comparatively large increases in acidity. At the end of the experiment addition of 2 grams of HCl causes about as much increase in acidity as had the entire 318 grams previously introduced. Henderson says of the values in the above table: "From the beginning of the experiment until almost 250 grams of hydrochloric acid have been added, neither alkalinity nor acidity is double in intensity the values which obtain in a perfectly neutral solution. . . . Such close approach to neutrality can be attained with pure water only after elaborate and very difficult purification, yet in the presence of carbonic acid it is the natural condition."

The dissociation of phosphates, which has already been described, serves with equal efficiency to preserve a solution in a state close to neutrality; but in the actual H ion regulation of the animal body phosphates play a subordinate rôle. This is due to the fact that they are not nearly so abundant as bicarbonates and, in addition, their elimination must occur by the relatively slow process of kidney secretion.

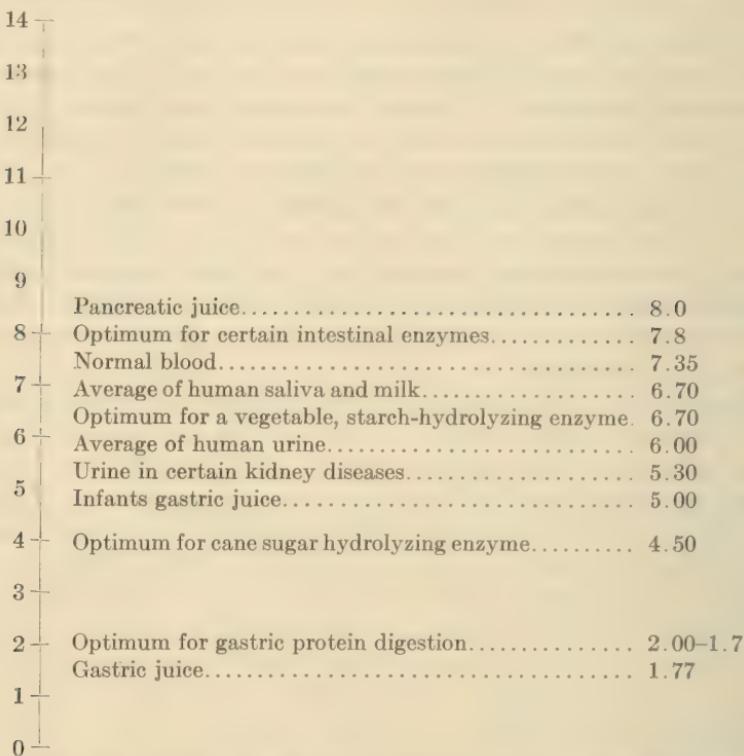
Another interesting fact was established by Henderson. Of a large number of weak acids, tested to find the amounts of alkali required to produce a given amount of change in the C_H of their solutions, NaH_2PO_4 and H_2CO_3 required larger quantities than all others, save H_2S . In other words, the buffer substances of the blood are among the very most effective that could be found.

Proteins and the very small quantities of amino-acids in the liquid part of the blood also show "buffer" action but they too are overshadowed in their effect by the bicarbonate. In protoplasm itself, where hydrogen ion concentrations have not been very successfully studied, neutrality is doubtless regulated to a high degree of perfection. Here, it is quite probable that proteins are an important agent. Hemoglobin in the red corpuscles has a buffer action that regulates the C_H not only of the corpuscles but indirectly of the whole blood (Chap. XX). Not only may proteins regulate hydrogen ion concentration, but their own physico-chemical behavior is very sensitively modified by changes in reaction. This matter will be considered in some detail in Chap. X.

Finally, let us take one more illustration to emphasize the effectiveness of the buffer regulation of neutrality in the blood. If to blood, containing the same proportion of carbon dioxide that it has in the body and kept under an atmosphere containing carbon dioxide sufficient to exert the same pressure that it would in the air of the lungs, we add $\frac{N}{20} \text{ HCl}$, we should find that the quantity of acid required to bring 100 cc. of blood to the condition of approximate neutrality that occurs just before death from acidosis would be no less than 25 cc. This represents a change of pH from about 7.35 to about 7.00. The same quantity of tap water of the same pH as blood would change by much more than this amount upon the addition of a single drop of the same acid. If a human body, containing 5,500 cc. of blood in the circulation, were to suffer the same change in C_H of the blood the equivalent of over 1,300 cc. of $\frac{N}{20} \text{ HCl}$ would have to be added to it. No wonder we regard acidosis as a sign of marked derangement in body metabolism.

The average concentration of hydrogen ions in a number of biological fluids and some other pH values of physiological interest are represented upon a scale below.

SCALE OF pH VALUES



DYNAMIC EQUILIBRIUM IN PHYSIOLOGICAL PROCESSES

The Study of Equilibria in Chemistry.—Equilibrium in chemical reactions in general and in electrolytic dissociation in particular as discussed in preceding pages suggests the fundamental significance of adjustments between opposing forces. For example, events tending to cause production of hydrogen ions are constantly met in the living organism by conditions tending to prevent their dissociation. Concentration of hydrogen ions in body fluids is, to be sure, a constantly changing quantity and the amount of its change is different in different parts of an organism; but in any case, it is only a small fluctuation about a mean value to which it must come back so soon as the balance of opposing forces is restored. Recognition of this principle in biochemistry has been slow. But the science of physics has long recognized that manifestations of kinetic energy can occur only when the equilibrium between opposing forces is disturbed. An elevated weight does not stay up because there is nothing tending to pull it down but merely because the forces of gravitation are balanced by those which support the weight. The helpfulness of this point of view as an aid to the development of physical science is very apparent to one who has studied resistances as a measure of strength of an electric current. This viewpoint rests upon

the principle of energetics namely: Potential energy represents forces in equilibrium while kinetic energy only exists when a balance of forces is disturbed. This is a necessary assumption if we accept the law of the conservation of energy. It is all the more surprising, then, that chemistry, although tacitly recognizing the law, was so late in applying the idea of equilibrium to its phenomena. This backwardness was due, in part, to the conception that while physics was the study of energy, chemistry was the study of matter. The chemist, separating matter from energy, tried to account for the reactions of energy upon matter. Although such explanations are adequate in many aspects of chemistry, yet to account for other aspects, for example, the reactivity of metallic sodium in contrast to the comparative quiescence of metallic gold, the chemist had to postulate a special form of energy—chemical energy. It was not then known that all matter is merely the region within which exist certain groupings of electrical forces. The electrical character of ion equilibria follows from the very definition of an ion as an electrically charged particle, but the newer knowledge of the electronic structure of all matter has brought out a larger truth: Every type of chemical reaction is, in the last analysis, merely a rearrangement of equilibria between electrical forces. Chemistry therefore is just as surely based upon energetics as is physics itself.

Another circumstance has greatly aided in newer developments of chemistry. The diversity and the accuracy of its quantitative methods have been distinctly increased. It is now possible to study the velocity of a chemical reaction as Newton once studied the velocity of a falling body. Chemistry like every other aspect of science began with merely qualitative observations. They fed it during infancy and childhood. Only quantitative information, ever more and more exact, could insure its continued growth.

The Difficulties of the Study of Equilibria in Biochemistry.—General chemistry has now reached the stage where the concept of equilibria is used to explain its phenomena, and Biochemistry has not lagged behind in the use of this illuminating idea. The physiologist, however, must encounter some very serious difficulties. Since these have not discouraged physiological research but have, indeed, been a stimulating challenge to work of especial interest, their formulation at this point will help to a more sympathetic understanding of the problems to be considered in subsequent chapters.

The nature of the difficulties is indicated by a specific problem: How is sugar oxidized? At once comes the question: Does this mean oxidized in laboratory glassware or in the human body? A thorough but physiologically inexperienced mechanist may say, "It makes no difference. The process is the same in either case." Or, leaning to a vitalistic philosophy, one may say:

The two are entirely different since whatever happens in the organism occurs under the influence of living forces—vital energy—or something of that sort. No information from the test tube can explain conditions in life.

There is, however, another point of view. It may be suggested by contrasting purely chemical processes with physiological ones. The chemist, to find how sugar is oxidized, will try the reaction in a test tube or its equivalent. He will have a complex problem because the process is not a simple chemical reaction but takes place in a series of separate ones. The numerous intermediary products formed before sugar is completely oxidized to $\text{CO}_2 + \text{H}_2\text{O}$ have perhaps not all been even identified. Their identification is difficult. But the velocity of their production and disappearance during oxidation is a still more difficult matter to determine. A series of reactions must be followed. Most of them are reversible and each one has a characteristic velocity. They will be discussed in Chap. XXI. If we represent them schematically thus:



we shall see how any one step might well be followed if we could isolate it from the others; but constituting a link in a chain, each reaction is really dependent on all the others. The several reactions may be very differently affected by slight changes in conditions: CH_4 , temperature, oxygen tension, etc. Only when one knew the equilibrium constants of each one of the separate reactions and how they were affected by changing conditions could one satisfactorily understand the process, or explain why certain combinations of products in certain quantities resulted from stoppage of the chain of events before it reached completion. Just such explanations are demanded by the physiologist who wants to know why sugar is not oxidized in the body during diabetes. He must first know how it is normally oxidized. But how much more difficult is his problem than that of the chemist! The latter working in the laboratory can largely control conditions. Temperature, concentration of the sugar solution, its pH, the oxygen tension, etc. may be regulated though probably no better than they are in the healthy human body; but in one respect the chemist has a great simplification of his problem. He can work with purified materials of a known composition and exclude interfering substances. The physiologist, on the other hand, must find out what happens in the body where many additional complexities are involved. In addition to the agencies present in all cells for the purpose of oxidizing such substances as sugar, there are in the blood no less than five products of as many different organs of the body which have been shown to affect all sugar metabolism. In addition, there are reactions in which sugar may be changed to glycogen or to a fat or possibly other products. Any one or more of these disturbing factors—and this is by no means a complete list—may disturb the equilibrium of some one or

more of the numerous reactions involved in sugar oxidation. Is it any wonder, then, that after all the numerous volumes already written on the story of sugar metabolism, there are still very interesting sequels to be produced? The wonder is that it has been possible to write so large a part of the tale.

The complexities involved in sugar metabolism are no greater than those which accompany any other phase of living activity; so that an attempt to consider any physiological event isolated from the accompanying ones is unsatisfactory. Recall, for example, the influence of concentration of calcium and potassium ions upon the heart beat. Seemingly, calcium starts the contraction process which in turn liberates potassium ions which in their turn cause the relaxation process. Equilibrium is again disturbed by some process causing an increase of calcium ions and the heart cycle is repeated. This very simple explanation is quite inadequate. Even if we could identify some reactions in heart tissue cells which possessed such an equilibrium that it was made to oscillate first in one direction by increased concentration of calcium ions and then in the opposite one by an increased concentration of potassium ions, we should still be very far from the end of the story. Other factors must be taken into account. The concentration of hydrogen ions, for one thing, enters into the process since very slight changes in pH markedly affect the heart. Moreover, oxidation—with all its complications—is involved somewhere in the chain of events. This is apparent because sugar, or its equivalent, and oxygen are required to maintain heart activity. Many other factors are concerned, factors such as nerve impulses and the influences of numerous substances that may be brought to the heart from other parts of the body. Their detailed discussion belongs with a study of the physiology of the heart and will therefore be deferred. Enough has been said however to show that the activities of the heart are correlated not only among themselves but also to various other bodily activities.

Dynamic Equilibrium.—The heart responds delicately to these many influences, because its activity is the sum total of a great many reactions each of which goes reversibly back and forth about an equilibrium that, while tending to remain constant is very easily disturbed. Because it represents, in each case, no static conditions, but rather a balanced action of opposing forces it is called *dynamic equilibrium* or *mobile equilibrium*.

Reversibility of Life Processes.—A pronounced characteristic of living things is the reversible nature of the responses to environmental conditions. When a plant responds to light by photosynthesis, the active cells are so adjusted that their products are continually removed so as to make way for continuation of the process or else the reactions are reversed, so that in any case the plant cell need vary in its chemical composition only around a certain normal or average value. When a muscle

responds to a nerve impulse by contracting, it is able to so restore itself, largely by reversion of its chemical processes, that it is ready for another contraction very shortly after relaxation. When a nerve conducts an impulse, it is so rapidly restored to the condition prevailing before the impulse occurred, that it is ready within a few thousandths of a second to receive and transmit another stimulus. In short, every living mechanism is able in some manner to compensate, internally, for every physiological activity. Certain artificial machines are also so constructed as to show internal self-compensatory adjustments. An instructive example is found in the charging of the storage battery of an automobile by the generator which is run by the motor which is fired by the current from the battery itself. The charging device is self-limited, in some machines, so that when the battery is sufficiently charged, the charging current is cut off automatically. This illustration is especially helpful because many of the compensatory adjustments of living organisms are self-limited in that they operate to produce a given result only up to a certain limit which is fixed by the intervention of some other process. For example, sugar oxidation in response to sugar concentration in animal tissues exhibits a self-limitation; for while an increase in the concentration of glucose in the tissues tends of itself to cause an increased rate of sugar oxidation, it can do so only up to a certain limit beyond which further increases in glucose concentration cause storage of glycogen and fat. Although comparisons between machinery and the living mechanism can be made, the latter has finer adjustments, involving a greater number of reversible physico-chemical reactions, than are ever possessed by artificial machines. So characteristic are the compensatory adjustments of vital processes that a distinction between life and death is based upon them. Death may be defined physiologically as an irreversible change in living matter, a change for which the organism cannot compensate. To just the extent that any chemical, physical and physico-chemical changes in living matter occur in an irreversible manner, to just that extent protoplasm, suffering such changes, is to be regarded as partially dead. All *typical* processes of life, as distinguished from lethal processes, must be reversible.

Certain physiological activities are exceptional, in that they do not occur reversibly. For example, the oxidative reactions by which protoplasm develops energy for its activities, though potentially reversible, proceed under conditions that do not permit reversion; but so long as an adequate supply of food is maintained the protoplasm is nevertheless able to keep its chemical composition constant within narrow limits. This is comparable to the oxidation of fuel in a motor in that the process proceeds irreversibly, although movements of pistons and other parts are reversible. Another exception to physiological reversibility is obvious in the case of the biochemical changes characteristic of growth and structural

development and of senescence. These changes are lethal in the sense that they are nonreversible; so that even the youngest embryo of a higher plant or animal, having once started to grow, is already on the road to inevitable death. The familiar words, "In the midst of life, we are in death," are thus capable of being stated in terms of non-reversing chemical reactions.

Even the processes of senescence are reversible in some cases and under some conditions. This fact is demonstrated in the behavior of protozoa, such as *paramoecia*. These organisms ordinarily reproduce asexually by mere division. The rate at which they divide, under conditions of constant temperature and food supply, eventually suffers a decrease. This is taken to indicate senescent decline of vitality. But if at this stage two separate cells fuse together, the protoplasm of the resulting union is so rejuvenated that the initial optimal rate of cell division is restored.

Inasmuch as certain unicellular organisms, protozoa, yeasts, bacteria, have been observed to grow and divide alternately at a rate that remains constant under uniform environmental conditions for an indefinitely long period, the assumption that some types of protoplasm are potentially immortal appears to be justified.

This appears to indicate that some kinds of protoplasm have compensatory self-repairing powers that are perfect under favorable conditions of life. Their perfection can be explained on the basis of reversible reactions, all of which, whether physical, chemical or physico-chemical can come to a dynamic equilibrium from which they may be disturbed by suitable changes in the conditions that influence them, but to which they return because of compensatory reactions.

The Significance of Dynamic Equilibrium in Physiology.—The principle of dynamic equilibrium in reversible reactions is fundamental in all modern physiological conceptions. Because of this principle one can understand how living matter is responsive to changes in environmental conditions and yet can compensate for these change so as to maintain itself in a state of comparative uniformity of chemical and physical structure. In the light of this principle one can see how all the living cells within one organism are so correlated to each other that any event in any part of a complex plant or animal may affect physiological processes in any or possibly every other part. An organism, therefore, behaves as a unit so that in many respects we find it impossible to explain satisfactorily the behavior of one organ such as the liver, or even a single brain cell, without considering its interactions with many other structures and, in some cases, with every other cell of the organism. The older conception of physiology as the study of the functions of separate organs must be modified to recognize the fact that many organic functions cannot be studied satisfactorily as isolated activities but must be investigated in relation to the behavior of the organism as a whole.

The principle of dynamic equilibrium has given a clew to the previously mystifying complexity of life. It is the unifying principle which ties all parts of an organism together.

Use of the idea of dynamic equilibria makes it no longer necessary to fall back upon a hopelessly unproveable assumption of the existance of an entelechy, a vital directing spirit, a "biotic form of energy" or some other force called by a sophisticated name designed to camouflage the lack of a theory sufficiently definite to be capable of experimental testing. But accepting the idea of mobile equilibrium, why are we still so very far from a complete interpretation of life phenomena? Because the living mechanism is so extremely complex in physico-chemical organization that we cannot as yet even enumerate the many delicate compensatory adjustments about the countless interrelated equilibria. Only intensive and ever more accurately quantitative study of metabolism in all its phases can throw light upon the problem. A mechanistic interpretation of life can be attained only as fast as all scientific knowledge advances. A comparatively simple mechanism, a watch, was a living thing possessed of a devil in the mind of the savage redskin. An automobile with self-charging storage batteries, automatic temperature regulating devices, an automatic timing regulator and a number of other self-regulating adjustments might well seem to educated people of the eighteenth century to be possessed of a devil or at least of some unrecognizable directing spirit other than that which prevails in the universe as a whole. Possibly a newspaper press or any other of the marvellous automatic machines used in modern manufacturing industries would be still more mystifying. Yet to the student of physics and chemistry, as we now know them, a printing press is no more weird in principle than the simplest watch ever devised. The automatic machine is merely more complex because it displays many more mobile equilibria to give it compensatory automatic adjustments. Though we of the present generation believe that we understand the fundamental principle, yet so far as the artificial production of living matter is concerned we are probably no nearer its realization now than was Newton to the invention of an aeroplane of the present day type—we may not be as near as that. But just as Newton, Faraday and others of their time unlocked the outer doors of the secret places of physical science; so the physiological investigators of the past 35 years have found the key which, we hope, will forever insure to us the satisfaction that neither superstition nor honest philosophic reasoning shall lock away from us the secrets of life.

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CHAPTER VIII

THE VITAMINS

The Discovery of Vitamins.—Previous to about 1912, the actual nutritive requirements of animals were generally supposed to comprise nothing more than proteins, fats, carbohydrates and the correct assortment of inorganic salts. The discovery of the fact that still other requirements must be met was the culmination of previously puzzling observations made at all periods of human history.

Writings of the ancient Greeks show that human scurvy, now attributed to deficient diet, was then known, but not understood. During the middle ages, the records of the Crusaders showed the occurrence of this disease and attributed it to something injurious in the diet rather than to the lack of some requirement. Indeed, all armies, of all times have been liable to scurvy. It has appeared whenever extended campaigning or other conditions have limited the opportunity to get fresh and varied foods. The same has been true for navies and all ships crews on long voyages. Captain Cook, the English explorer and trader, was one of the first to recognize the value of fresh food as protection against scurvy. His extended voyage, begun in 1772, has become famous through the fact that no scurvy appeared among his crew, although the voyage lasted over three years and covered vast stretches of the South Pacific and South Atlantic oceans. Such a conquest of this ancient scourge was unknown up to that time. Captain Cook attributed his success to liberal use of such fresh fruits and vegetables as could be obtained and of sauerkraut and barley. Partly on account of these observations and partly because of other similar ones, regulations for the British navy have required, since 1795, that all ships crews be supplied with those fruit juices and vegetables which prevent scurvy. Not only armies and navies, but exploring expeditions and camps of lumbermen and other isolated persons have suffered from scurvy. Although this disease had thus indicated, during all centuries of recorded history, that some peculiar food substance was required, recognition of the vitamin character of the substance which prevents scurvy came only in recent years (1919–20). Previous theories had tried to account for scurvy as caused by the lack of correct adjustment of inorganic salts of the diet to nutritive requirements or as due to poisoning that resulted from bacterial action in the intestine. But even-

tually, the combined observations of many investigators proved that this disease could be prevented only by eating those foods which contain the so-called antiscorbutic (scurvy-preventing) vitamin.

A similar story is found in the history of the peculiar disease, called beri-beri, which has also been one of the ancient scourges of certain races, although definite records, dating as far back as those of scurvy, appear to be confined to Chinese writings. Like scurvy, beri-beri is a disease with definite and, in late stages, unmistakable symptoms. Either disease is rapidly fatal if not counteracted by the appropriate foods. Beri-beri occurred chiefly in Asiatic countries, India, China and Japan, in short, in those countries where rice was the staple article of food. But the disease also raged among ships crews and other persons subsisting on a restricted diet. The unquestioned prevalence of the disease among rice-eating people led to the theory that fungus or bacterial growth in rice might be the cause. Indications of food deficiency as the cause were not lacking, however. The Japanese found, for example, that when in 1884 they increased the variety of foods in sailors' rations, the ravages of beri-beri in the navy were checked and that a ration, including meat and other animal foods, eradicated beri-beri from the naval forces of Japan during the Russo-Japanese war. This triumph over the disease could not be attributed to the removal of something from the diet as the customary foods, chiefly rice, were still generously supplied. The result must have been due to the additions that were made to the diet. The disease, moreover, became obviously more prevalent among civil populations in those localities where the use of polished rice (milled so as to remove the outer hulls and most of the embryo) replaced the custom of eating whole rice.

The first really significant discovery on this subject was made by Eijkman, a Dutch investigator, who showed, in 1897, that chickens, fed exclusively on polished rice, developed certain symptoms, including paralysis, that were typical of beri-beri. These symptoms appeared in the course of two or three weeks on the diet, but were rapidly cured by feeding whole rice or rice polishings. This discovery, however, did not receive sufficient attention to guide further research into an immediate solution of the beri-beri problem.

In 1906, Hopkins, an English investigator, obtained significant results from feeding experiments with rats. He showed that a diet, composed of isolated, purified foods, was inadequate for growth or even for life, although it contained proteins, fats and carbohydrates in correct proportions and included all the inorganic constituents known to be required. Hopkins found that a very small quantity of milk (3 cc. per day) added to this faulty synthetic diet rendered it entirely adequate for maintaining healthy growth of rats. He recognized the fact that none of the known constituents of milk could produce this effect and so attributed his result

to the presence in milk of some previously unrecognized nutritive requirements which he named "accessory food factors."

In 1911, Funk, then in London, discovered that an alcoholic extract of rice polishings yielded a crystalline substance which, in extraordinarily small amounts, could cure the morbid condition of birds fed on polished rice. At almost the same time Susuki and his co-workers in Japan published substantially the same discovery. Funk's preparation contained nitrogen in a form that gave the reactions characteristic of amines. Although he did not isolate the substance in sufficiently pure form to really identify it, he believed that he had at least shown it to be an amine and as it was necessary for life, proposed to call it vitamine. He suggested what has since been known as Funk's vitamine hypothesis, namely, that the diseases curable by adding something to the diet, such diseases as scurvy, beri-beri, rickets and pellagra, are each caused by the lack of some specific requirement comparable to his vitamine. Subsequent research has substantiated his hypothesis in some particulars though not in all respects.

The year 1911 also marked further significant advances in the science of nutrition. At that time, Osborne and Mendel published the results of a series of researches primarily designed to study the relative nutritive value of different proteins. Their method consisted essentially in observations on the rate of growth, as shown by weight curves, of white rats maintained upon synthetic diets, composed of purified food stuffs. The significance of these researches for protein chemistry, showing the animal requirements for the so-called complete proteins, was explained above (p. 30). These investigators soon found, however, as had Hopkins, that no strictly synthetic combination could keep rats alive. In using milk to supply the unknown food factors, they further found that two apparently different unknowns were required. One of these was present in the whey of clotted skimmed milk, that is, in the milk constituents that remain after the fats are removed by centrifuging and the proteins by coagulation (p. 16). When the whey, thus obtained, was evaporated to dryness, the residue, which they called protein-free milk, contained lactose, salts and one of the accessory food factors. The other factor, which they found to be equally necessary for successful growth, was found in the milk fat; so that butter was included in the ration of all rats successfully reared. The peculiar nutritive potency of butter had been previously indicated by the similar observations of Stepp (1909), although he believed he had thus shown the animal requirement for certain lipoids rather than for some substance of unknown character.

McCollum and his co-workers had meanwhile undertaken an elaborate analysis of the dietary values of certain staple foods. They, too, arrived at the same conclusions as did Osborne and Mendel and were further able to show that, the accessory food factor contained in milk whey was

identical with Funk's anti-beri-beri vitamine, while the one contained in milk fat was an equally indispensable factor whose deficiency in the diet caused disease symptoms quite as unique and characteristic as those due to lack of the so-called vitamine. McCollum proposed to call these substances the fat-soluble A and water-soluble B dietary factors, thus designating them by the only chemical properties which they were surely proved to possess.

The name vitamine has been criticized because Funk's beri-beri-preventing substance has not yet been proved to be an amine while the fat-soluble A factor does not even contain nitrogen and therefore cannot be an amine. But as vitamine is a more convenient term than accessory food factors or any other name that has been proposed, its use has become general and too widespread to be easily changed. Drummond has proposed to designate these accessory food substances by the word vitamins. This spelling is recommended because of the convention among organic chemists of designating a neutral substance of unknown chemical constitution by a name ending in "-in." As the chemical nature of these substances is still unknown the several ones may be appropriately called vitamin A, vitamin B, etc. Further description will be now given for each of the three that have been proved to be indispensable and for others that have been postulated as distinct dietary requisites.

Vitamin A.—Other names that have been used to designate vitamin A are: Fat-soluble A, dietary factor A, fat-soluble vitamin and the growth-promoting accessory food factor.

Our knowledge about this substance, indeed about each of the vitamins, is chiefly confined to the effects of its lack in the diet. These effects are: (1) Failure to grow; the younger the animal, the more pronounced is the requirement for a generous supply of this vitamin in order to maintain the normal rate of growth; (2) failure of the normal secretion of the glands of the eyelid, resulting in a condition of dry eyes (xerophthalmia), which favors bacterial infection to such an extent that a large proportion of animals, lacking a supply of this vitamin, have sore, pus-filled eyes, and a conjunctivitis that eventually causes total, permanent blindness, if the vitamin deficiency is not corrected in time (Fig. 37); (3) a similar failure of secretion of the sebaceous glands of the skin, so that the animal's coat becomes shaggy and generally unkempt in appearance. The failure of growth is further aggravated by the loss of appetite in the absence of this vitamin. This condition causes marked loss of weight in adult animals lacking a supply of vitamin A, and their general malnourished appearance is apparent. A sufficiently prolonged deprivation of vitamin A is fatal for all species of animals that have been tested. A chart of growth curves of rats as shown in Fig. 38, illustrates the typical effect of a lack of this vitamin,

The occurrence of vitamin A in natural foods shows that it tends to accompany certain fats, though by no means all. Of animal fats, cod liver oil is probably the richest in this vitamin. All kinds of liver fats contain much of it. Butter and the oily fats of egg yolks are also richly supplied. Fats from the adipose tissue of animals contain only a very little and may be entirely lacking in it, in some cases. In general, then, meats are not an important source of this vitamin. Among plant fats, the



FIG. 37.—Photographs to show the need of vitamin A. The dogs in the two upper pictures show prostration and severe xerophthalmia as a result of a diet deficient in vitamin A. The lower picture shows one of the same animals after being treated 10 days with 20 cc. of cod liver oil daily in addition to the previous diet. (From Steenbock, Nelson and Hart, *Amer. J. Physiol.*, 1921).

majority are conspicuously deficient. All the plant oils from nuts, seeds etc. do not contain this vitamin. The green leaf parts of plants, however, contain it abundantly. Spinach, clover and alfalfa have been especially tested, but probably all leaves and grass contain some, at least. One is not accustomed to think of leaves as fat-containing structures and the amount extracted by ether is, indeed, small. Moreover, a pure ether extract of leaves does not contain the vitamin. It is necessary

to treat the plant first with alcohol and subsequently extract with ether or to use an alcohol-ether mixture in order to obtain fatty material that is potent as a vitamin A food. The young sprouts of plants contain this vitamin although the grains from which they arise contain only traces. Fruits and the roots and tubers of plants also contain only traces. The occurrence of vitamin A tends to be parallel to that of the yellow pigment, carotin, in a remarkable way. Carrots and sweet potatoes, for example, contain the vitamin and are rich in carotin; but white potatoes lack the vitamin. Yellow, carotin-containing corn has vitamin A, while white corn lacks it. The rich supply in green leaves would suggest some association between the vitamin and chlorophyll, though carotin is also found in green leaves; but as explained in Chap. I, synthesis of this vitamin does not require the presence of chlorophyll or even the action of light. The major sources of vitamin A, then, are liver oils, butter, egg yolks and green leaf parts of plants. Minor sources are the other animal fats and the carotin-containing vegetables.

The close association with carotin suggests that the chemical nature of vitamin A is similar to that of the pigment. Information about its chemical character is, however, meagre and chiefly negative. This scarcity of knowledge is due to the small quantity of the substance present in even the richest sources and to the fact that no chemical or other quick test for it has been found. Laborious and time-consuming feeding experiments are the only means available for detecting the presence or absence of this or any vitamin. This vitamin cannot be carotin itself as certain nearly colorless oils contain it while many carotin-rich preparations are practically free from it. It is soluble in alcohol and ether and is very slightly soluble in water so that it is not entirely lacking in skimmed milk. It can be prepared in concentrated form by evaporation of its solutions. It is destroyed by oxidation, especially at high temperature and is much more susceptible to oxidative destruction after extraction from its natural source than when in the tissues. This fact signifies that the vitamin naturally occurs in combination with something else, presumably a protein or a lipoid. This probability is further indicated by the fact that tissues must be first treated with alcohol if the vitamin is to be made soluble in ether. Vitamin A does not contain nitrogen or phosphorus because highly purified butter-fat, freed from these elements, still contains the vitamin. It is not a fat or a lipoid because saponification does not destroy it. Since all nitrogen and phosphorus compounds, fats, lipoids and certain pigments are excluded, investigations leading to its chemical identification are at least narrowed down.

Information concerning the manner in which this vitamin produces its physiological effects is meagre. Like all vitamins it acts in exceedingly small amounts. The proportion of it in butter fat must be very small, since known constituents add up to approximately 100 per cent;

yet when only 5 per cent of rat's food consists of butter and all other food constituents are devoid of vitamin A, the animal grows normally and maintains good health. As little as 0.1 gram of butter per rat per day has apparently been adequate in some instances. A more generous supply permits storage of the vitamin in liver and other fat-containing tissues; so that animals can withstand deprivation of vitamin A, after a diet rich in milk or butter, much longer than do those previously fed a bare

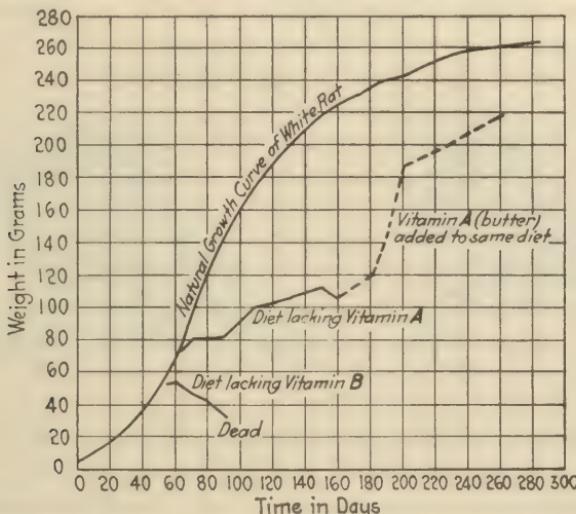


FIG. 38.—Growth curves to show the effects of lack of vitamins A and B on white rats. The lower curve shows that lack of vitamin B may cause failure to grow or loss of weight sooner even than does the lack of vitamin A.

minimum of the vitamin. The frequent occurrence of eye infection as a result of the lack of this requirement suggests that it has some relation to resistance to infection. All attempts to prove this point by the methods of immunology have failed, however, to reveal any especial protective action in the ordinary sense. Several investigators have reported a high incidence of pneumonia and other lung infections among animals lacking the vitamin. Loss of appetite has been referred to. Diarrhea has been frequently observed. But in general, the physiological action of vitamin A remains to be investigated.

The chances that human beings will suffer from a lack of this vitamin are not large under ordinary conditions; but, under abnormal conditions of food supply, this lack has undoubtedly been felt. During the years 1917-18, when the price of milk and butter was very high, numerous cases of stunted growth and serious eye disease, often resulting in blindness, occurred among children of poorer classes in Japan and several European cities. Especially numerous cases were reported in Vienna where dairy supplies were notably deficient. Many of these cases were cured by an improved milk supply and in Denmark, an improved dis-

tribution of butter, insured by government action, resulted in complete disappearance of the trouble. Lactation has also been regarded as likely to reveal effects of this deficiency. The nursing mother requires a generous supply of vitamin A, owing to the selective action of the mammary gland, which secretes this requirement into the milk, even at the expense of the maternal organism.

Vitamin B.—Other names for vitamin B are water-soluble B, dietary factor B, water-soluble vitamin, beri-beri-preventing vitamin, and anti-neuritic vitamin. As explained above, this was the first of the vitamins to be named as such and to be recognized as something distinct from previously known food requirements.

The chief effects of its lack are failure to grow and beri-beri in human beings or its equivalent, polyneuritis, in animals. Some writers have questioned whether all these effects are really due to lack of the same vitamin; because, when all constituents of the diet are adequate, save vitamin B, addition of milk appears to further growth better than it prevents polyneuritis. For a long list of foods, however, growth promoting power and protection against polyneuritis run parallel; so that, in the absence of more information, the simpler explanation of McCollum attributing both effects to lack of the same substance, has been generally accepted. Beri-beri is also called polyneuritis because its most marked symptom is a general inflammation of the nerves resulting in paralysis of the limbs and sometimes in nearly total paralysis, though death usually intervenes before that stage is reached. Early symptoms are loss of appetite, diarrhea, failure of digestion, tendency to anemia, emaciation and occasionally oedematous swelling of the skin. Later stages show the degeneration of numerous organs accompanying the neuritis. In some cases, marked degeneration of testes or ovaries and sterility have been noted. The immediate cause of death from this lack is commonly said to be heart failure. A growth curve, showing the effects of deprivation on rats, is given in Fig. 38. Growth failure, owing to lack of vitamin B, is even more pronounced than in the absence of vitamin A.

The physiological action of vitamin B appears to be exerted more particularly upon the nervous system. Since neuritis is caused by this lack and rapidly cured by supplying the vitamin, its rôle in nerve functioning is clearly indicated. Truly spectacular effects are produced. A bird in the rather advanced stage of paralysis—almost complete loss of the use of the limbs (Fig. 39), may show an apparently complete recovery in three to six hours after eating a small quantity of vitamin-containing food. Why birds are more quickly susceptible to this particular deficiency than are other laboratory animals and why they recover so rapidly, when the lack is supplied, has not been explained. Except for the rapidity of the action the effects in birds are comparable to those in man and other animals. Marked degeneration of peripheral nerves has been frequently

found at autopsy of animals dying from this lack. The rapidity of the onset of symptoms of its lack indicates the poor capacity of the animal body to store vitamin B.

The marked failure of digestion has been shown to be due to poor secretion of the digestive juices. This does not necessarily indicate that the vitamin exerts a direct influence on secreting functions of the digestive glands. Indeed, experiments have shown that injection of vitamin B preparations does not directly stimulate secretion of pancreatic juice, the



FIG. 39.—Polyneuritis cured by vitamin B. The picture on the left shows a bird in advanced stage of paralysis as the result of a diet deficient in vitamin B. The other picture shows the same bird 3 hours after the administration of 4 mgr. of Funk's yeast vitamine preparation. (From Funk "The Vitamines," Williams and Wilkins Co.)

most important digestive juice. As all digestive secretion is either directly or indirectly dependent on nerve stimuli, the failure of nerve functioning may alone account for the digestive failure.

The occurrence of this vitamin in natural foods is widespread. Owing to the limited capacity of animals for storing it, vitamin B is not abundant in foods of animal origin. Even milk, that nearly perfect food, is not one of the rich sources; though, with the exception of eggs and possibly brains, it is probably richer in this vitamin than are any other foods of animal origin. As all animal tissues contain a little, carnivores are adequately supplied. In plants, however, the supply is abundant and pretty uniformly distributed in all tissues. The richest stores that have been discovered are in yeast and in the embryo of grains—especially wheat embryo. Funk's original discovery of the material in rice polishings indicates its presence in the outer hull of the grain; but as the polishings also contain some of the embryos, the latter are probably the chief contributors to this yield. The pericarp (hulls) of other grains are a fair, but not especially rich source of the vitamin. The typical storage parts of plants, those parts that are rich in starch, are almost devoid of it; so that refined milled flour, which is minus both hulls and embryo, is conspicuously deficient. Nuts and fruits are generally abundant sources and, though varying in the quantity contained, never lack it entirely. The green parts of plants, spinach, clover, alfalfa, etc.,

and the root parts, potatoes, beets, onions, carrots, etc., are all fairly rich. This wide distribution of the vitamin among the foodstuffs shows why its lack is seldom encountered by man or domestic animals. Only those diets, very largely composed of some one refined product, like polished rice or wheat flour, could fail to supply at least a fair quantity of this requisite.

Especially elaborate investigations have been directed toward the chemical identification of vitamin B; but as results have been conflicting, the problem is still unsolved. The crystalline preparation, as made by Funk, has been duplicated by other investigators. Williams believes his preparations to be related to the betains, a group of nitrogen-containing bases. But quantities, sufficient for chemical identification have not been obtained. In fact, it has not yet been proved that any of the preparations are unit substances. All agree that the substance is labile, that is, capable of rapid and spontaneous change when in supposedly pure form. It is soluble in alcohol and water and can be easily removed from plant and animal structures by either of these solvents, although 70 per cent alcohol is most frequently employed. Contrary to the earlier views on the subject, this vitamin is now regarded as soluble in fats, after it has been separated from its natural sources. Thus the distinction between fat-soluble and water-soluble vitamins is not so sharp as originally supposed. Vitamin B, like A, probably occurs in some combination, because it is not readily destroyed by heating the foods that contain it. It is much more susceptible to destruction in alkaline than in acid or neutral solution. It is precipitated by the alkaloidal reagents, such as pieric acid and by metallic salts, such as mercuric chloride. It is adsorbed by fuller's earth (Lloyd's reagent) when its neutral or acid solutions are mixed with this reagent. Subsequent treatment of the earth with dilute alkali redissolves the vitamin which can then be recovered, if the alkaline solution is quickly neutralized. This procedure has furnished the means for separation of vitamin B from vitamin C. All these facts and some others that have been established promise a solution of the problem of the chemical nature of vitamin B.

Vitamin C.—This vitamin is also called water-soluble C, the scurvy-preventing food factor and the antiscorbutic vitamin.

The effects of its lack include, as in the case of the other vitamins, failure to grow. Babies, suffering from a deficiency but not a complete lack of vitamin C, may show no symptoms other than stationary weight. Occasionally other symptoms appear even though growth is nearly normal in rate. The chief effects, however, are those included under the term scurvy. Among the symptoms of scurvy, are extravasations of blood, appearing as red spots under the skin and forming hidden bleeding places in any of the internal organs or the muscles and particularly in periosteum

of the large bones. Bleeding and soreness of the gums, accompanied by marked decay of the teeth, with consequent prurient infections of the mouth, constitute one of the most prominent symptoms. The teeth are very apt to fall out. Another particularly noticeable feature is the extreme soreness of the joints so that movement or even touching of the joints is very painful. Accompanying this condition, is a noticeable change in the ends of the bones which become very fragile and easily broken. Swellings on the ribs, similar to those which are characteristic of rickets, also occur. X-ray pictures of the bones, especially of the joints, give one of the surest means of diagnosis of the disease, since the changes that occur in them are distinguishable from those due to other diseases—even rickets. The digestion is markedly impaired. Constipation, followed by diarrhea is usual. The heart is often badly affected, showing hypertrophy and, later, degenerative changes which often cause sudden death of the victim at a time when the disease does not appear to be progressing rapidly.

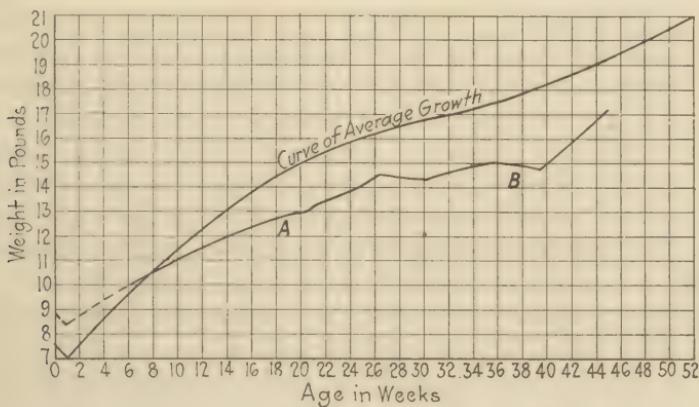


FIG. 40. —Weight curve of an infant to show the need of vitamin C. At A the child was weaned and placed on a diet deficient in antiscorbutics. At B a diagnosis of scurvy was made and the administration of orange juice begun. The subsequent upward trend of the growth curve is noticeable. (*Curve of average growth after Holt. Data for lower curve supplied by Dr. H. E. Utter.*)

Under modern conditions, scurvy is more apt to appear in children, especially infants, than in adults, because the latter are more apt to use scurvy-preventing foods. Though infantile scurvy was formerly described as a distinct disease, it is now generally regarded as the same thing as adult scurvy, only in a milder form. It is often due to an inadequate supply of vitamin C rather than to a complete lack of it. A weight chart, showing the distinctive effect of an antiscorbutic food on an infant, is given in Fig. 40.

Different species show markedly different susceptibility to scurvy. Guinea pigs are especially liable to it. They sometimes show severe

symptoms at the end of two weeks on a scurvy-producing diet and almost invariably succumb within four weeks. Rats, on the other hand, have never been known to show the disease even though their diet, during successive generations, is almost entirely lacking in all of the foods that protect guinea pigs from scurvy. This signifies either that rats do not require vitamin C or that they produce it in their own bodies. Feeding experiments, that employed rat livers as the only source of vitamin C, indicate that rats produce it. If this is really the case, it is the only instance, so far discovered, of vitamin production in animals. The prairie dog, a rodent (as are also rats and guinea pigs), appears to be as indifferent to lack of vitamin C as is the rat. The other animals, that have been tested, are sensitive to lack of vitamin C although less so than are guinea pigs. Man is perhaps second only to the guinea pig in susceptibility.

The chief sources of vitamin C are the fresh fruits and vegetables. The richest supplies that have been detected are in the juices of the citrus fruits, especially lemons and oranges. Tomatoes and cabbage are also very rich in vitamin C. Indeed, all fruits, leaf-parts of plants and all root vegetables are to be regarded as useful sources. Potatoes, though not conspicuously rich in vitamin C, are consumed in such large quantities, compared to other antiscorbutics, that they are often spoken of as the chief protection of the human race against scurvy. Statistics show that the incidence of scurvy has noticeably increased in several localities after a failure of the potato supply. Nuts and grains are very deficient in vitamin C, indeed, almost entirely lacking. Flour and other cereal foods, prepared by refining of grains have no antiscorbutic powers. Freshly germinated grains, such as barley malt, however, are fairly good antiscorbutics. This indicates that early stages of plant growth are accompanied by synthesis of vitamin C as well as of vitamin A. Foods of animal origin are never rich in vitamin C. Meat has a little antiscorbutic power and milk, in sufficient quantities, is adequate, as is clearly shown by the satisfactory growth of the nursing infant. Cow's milk, however, has been shown to vary in its antiscorbutic potency in accordance with the quality of the fodder. In summer, cows, on fresh pasturage, give milk of such potency that some 20 cc. per day are adequate to protect a guinea pig against scurvy; but during the winter, when cows are fed on hay and grain, their milk becomes progressively poorer in content of vitamin C, until 80 cc. in the daily ration of a guinea pig may fail to protect against scurvy. This indicates that the animal body can conserve a store of the vitamin and gradually pay it out in the milk. It also indicates the practical significance of maintaining a generous supply of antiscorbutic foods in the diet of nursing mothers.

Vitamin C is especially liable to be destroyed during heating or storage of food. This is the reason why the salted, canned, desiccated or otherwise

preserved food, constituting so large a part of the ration of ship's crews, soldiers, occupants of lumber camps, etc., do not furnish adequate antiscorbutics. The cause of this destruction is an oxidative reaction that is greatly hastened by rise of temperature and by alkalinity but is inhibited by acidity. The fact that the reaction is an oxidation is shown by the observation that hydrogen peroxide or ozone rapidly destroys the antiscorbutic power of a food, even without heating. The oxidative character of the reaction is further indicated by the failure of this destruction to occur when foods are heated in the absence of air or other source of oxygen. A very brief heating at 100°C. is less destructive than a prolonged heating at 80°C. By the use of these principles, it has been possible to prepare dried milk powder, dried orange juice, and desiccated vegetables in such manner that the antiscorbutic power is still retained. The sensitiveness of vitamin C to temperature has resulted in serious ravages of infantile scurvy where pasteurized milk was used as the only food of children. Recognition of this fact has given opportunity for an important advance in the science of child nutrition. Infantile scurvy should now be entirely eliminated by giving antiscorbutics to all children subsisting on pasteurized milk. For this purpose, orange juice is widely recommended; but less expensive materials are also available. The water in which potatoes have been boiled retains some antiscorbutic power. An infusion of barley malt is effective. Especially valuable is the juice of canned tomatoes. It is almost as effective as orange juice and is readily digested by most infants. The marked contrast between the rich content of vitamin C in canned tomatoes and the lack of it in other canned vegetables is explained by the acidity of tomatoes, which is sufficient to protect this vitamin from oxidative destruction during the heating processes of sterilization. Improved methods of pasteurization, designed to avoid oxidation bid fair to provide, in the future, a safe milk which has not lost its antiscorbutic power; but as even raw milk is sometimes low in its content of vitamin C, the use of orange juice or similar antiscorbutics in child feeding constitutes an indispensable safeguard.

Aside from its behavior to heat and other conditions influencing oxidation, the properties of vitamin C have been only partially investigated. It diffuses through a semipermeable parchment membrane. It is not adsorbed upon fuller's earth as is vitamin B. It is readily soluble in water. It is destroyed by strongly alkaline solutions even when oxygen is excluded and the temperature is kept low.

The significance of the recognition of vitamin C is even greater than that pertaining to A and B. Modern methods of refining foods have greatly increased the use of those that do not protect against scurvy. One has only to recall, for example, the increasing use of refined flour, sugar, canned and condensed milk and the multitude of cereal preparations to realize how large a proportion of our foods are lacking in antis-

corbutic power. Modern transportation and cold storage facilities, however, have enabled the human race to distribute fresh fruits and vegetables in a way that would have been amazing in previous centuries. The humblest corner store now displays, at all seasons of the year, the fruits of the tropics and a varied array of green and root vegetables. It is only necessary that the public be educated to an appreciation of the fact that fruits and salads are not a mere condiment or other food luxury, but have genuine and important nutritive values. There is no valid excuse at the present time, save ignorance, for the existence of scurvy when plant foods are available.

Are There Vitamins Other than A, B, and C?—Although the three vitamins described are the only ones whose existence has been definitely proved, several others are supposed to exist.

The problem of rickets, as explained in Chap. V, has been partially solved by showing that the disease involves a wrong adjustment of the proportion of calcium and of phosphates in the diet. Experiments have shown unquestionably that a sub-minimal supply of either of these requirements will produce a condition of rickets in experimental animals. Any condition which upsets the normal ratio of calcium to phosphates in the blood, appears to predispose to rickets. For example, ingestion of large amounts of magnesium or strontium salts, both of which are known to affect the excretion of calcium, have been found to produce a rachitic condition closely resembling typical rickets. Whether the disease be produced experimentally by calcium deficiency or by phosphate deficiency, it can be cured by cod liver oil. Feeding cod liver oil prevents rickets during the use of any diet which would otherwise produce it. Similar but less effective results can be obtained by feeding butter. These facts suggest that vitamin A might possibly be a protective agent against rickets. But even when butter is fed in amounts in excess of those required to supply enough vitamin A for purposes of growth and protection against eye disease, perfect protection against rickets is not obtained as it is with relatively small amounts of cod liver oil. The use of certain vitamin A preparations from plant sources does not appear to furnish any protection against rickets. Moreover, some investigators have even observed an aggravated condition of rickets as a result of adding small amounts of butter to a diet which had previously produced the disease. For these reasons, vitamin A can not be said to counteract rickets. It seems likely that some food factor, distinctly different from any of the recognized vitamins, is present in cod liver oil and is a specific anti-rachitic substance. This view has been advanced by McCollum, Simmonds, Shipley and Park on the basis of their extensive work on this subject. But in spite of much investigation, ideas on the cause and prevention of rickets are in an unsettled state. Certain observations have thrown much doubt upon the belief that any vitamin-like substance

is concerned in the prevention of rickets. These observations include the complete cure of rickets in children by mere exposure to sunshine without any alteration in the diet. Moreover, rats on a rickets-producing diet do not develop symptoms of the disease if they are exposed to sunshine. Similar effects in children have been obtained by exposure to ultra-violet rays from a mercury arc lamp without the use of sunshine. It is obvious that the relationship of vitamin A or any hypothetical vitamin substance to the production of rickets has not yet been proved.

Pellagra has also been suspected to be a deficiency disease in the sense that it might be due to a lack of some specific vitamin. Vitamin A was at one time regarded as requisite for prevention of pellagra. Some writers, notably Funk, have also suggested that some vitamin entirely different from the three known ones was concerned. Pellagra has been cured, however, by enrichment of the diet with complex foods such as milk, eggs, and meat; so that identification of any specific dietary factor as the curative agent has not been easy. The chief difficulty involved in such investigations is that the common laboratory animals do not develop typical pellagra symptoms under any conditions of malnourishment. The specific diagnostic symptom is a peculiar skin outbreak. The word pellagra, indeed, means rough skin (Italian). The peculiarity about it is its bilaterally symmetrical distribution. If an outbreak appears on one side of the neck, for example, it will also appear upon the corresponding area of the other side. There are other symptoms: Digestive disturbances, general loss of tone, etc. and the disease is fatal if a pellagra-producing diet is persisted in. These diets are typified by the food of the poorer classes in large sections of our southern states where pellagra up to recent years took a very large toll of lives. These people formerly subsisted chiefly on corn, canned preparations, wheat flour, sugar, molasses, corn syrup, bacon and salt pork. When potatoes and other fresh vegetables were freely eaten or when milk and other dairy foods or fresh meats or eggs were a part of the diet, pellagra did not appear. The pellagra-producing diet is liable to be deficient in several respects: Quality of proteins, supply of vitamin A, and supply of vitamin B. The curative foods that have been proved to be effective, make good all these deficiencies. Until pellagra can be produced in experimental animals by the use of rations deficient in only one respect and adequate with regard to all other known food requirements, the origin of pellagra cannot be determined. The nearest approach to this solution has been found apparently in experiments on monkeys. (Chick and Hume 1921.) These animals have been made to develop symptoms closely resembling those of human pellagra. The dietary deficiency that produced this effect was poor quality of protein food, however, and apparently had nothing to do with any lack of vitamins. In one series of experiments (Goldberger and Tanner, 1922) patients in the Georgia State Sanitarium

were not satisfactorily protected from pellagra by the addition of cod liver oil (rich in vitamins A and C) and of tomato juice (rich in vitamin B and C) to the well-known pellagra-producing diet. On the whole, then, the existence of a specific pellagra-preventing vitamin has not been proved nor have pellagra-preventing powers been proved for any of the known vitamins; while certain investigations indicate that the cause of pellagra is a diet whose proteins yield an inadequate supply of some of the requisite amino-acids.

The growth of yeast appears to be dependent upon a supply of some substance that is effective in very small amounts. Since this substance has not yet been identified, it is generally spoken of as a vitamin. Extensive researches have attempted to prove that the substance promoting yeast growth is identical with vitamin B. It has, indeed, been repeatedly shown that the anti-neuritic properties of many foods are proportional to their effectiveness in promoting yeast growth. A very small quantity of an extract of wheat embryo, for example, will markedly accelerate yeast growth. Wheat embryo, it will be recalled, is rich in vitamin B. But preparations, devoid of vitamin B, have been found in some cases to be highly efficient stimulants for yeast growth. This fact suggests that some vitamin-like substance, capable of stimulating yeast growth, commonly occurs along with vitamin B but is entirely distinct from it. The stimulating effect of minute quantities of vegetable extracts upon yeast growth was recognized by Wildiers before vitamins, as we now know them, had been discovered. He gave the name "bios" to the hypothetical substance that produced this effect. Bios may prove to be another member of the group of vitamins and to be as necessary for the life processes of yeast as are vitamins A, B and C to human life. As a matter of fact, however, the optimum requirements of the yeast plant for substances of known chemical composition, such as inorganic salts, nitrogen containing compounds, carbohydrates, etc., have not been satisfactorily determined. Until these are known, any attempts to study yeast requirements for "accessory food factors" are unsatisfactory because all vitamin containing preparations are complex mixtures.

The suggestion that bacteria are also dependent upon a supply of the recognized vitamins or of other hypothetical ones has also been made. But conclusive experiments to prove this point are lacking. It is possible that the symbiotic relationships between plants and bacteria, such, for example, as that shown between the clover plant and the root nodule colonies, is due to vitamin production in the green plant. On the other hand, it has been suggested that the beneficial effects upon plant growth, resulting from the presence of bacteria in soils, is not wholly due to bacterial decomposition of nitrogenous compounds so as to yield the important plant foods, nitrates and ammonium salts, but is also partly due to bacterial production of hypothetical substances, akin to vitamins,

that specifically favor plant growth. Such substances are given the provisional name, auximones. One of the observations that has led to this theory is that extracts of bacterially rotted peat stimulate the growth of seed plants.

From these several discussions, it is apparent that the existence of vitamins other than A, B and C still awaits satisfactory proof.

Practical Dietetics to Insure Adequate Vitamin Supplies.—The value of variety in the diet has always been recognized because of the consequent improvement in appetite and digestion and therefore in general nutritive condition. Vitamin discoveries have given a new and more definite meaning to the importance of food variety. It is only the monotonous diet that is liable to be lacking in a vitamin requirement. It is true that a monotonous diet might be so constructed as to be entirely adequate. It might even be composed of vegetable foods alone but would have to include, for human beings, a combination of cereal foods, root vegetables and leaf vegetables. It might even be composed entirely of foods of animal origin; but should, in that case, include a generous supply of milk and butter or eggs, in addition to meats. With any diet, the value of raw foods, in which vitamins have not been subjected to the destructive processes of cooking, preserving and refining, must continue to be emphasized until cooking and manufacturing processes, that do not permit oxidative destructions, are perfected and adopted.

The especial vitamin requirements of children demand particular attention to the quality of their diet. There is undoubted evidence from rat experiments and some evidence from experiments on other laboratory animals that, during the periods of more rapid growth, vitamin-rich foods are required in larger proportion in the diet than at other periods of life. Apparently the growth process itself partially uses up the available vitamin supply, so that maintenance of health demands an especial abundance of these requisites for the young animal.

The following table shows the relative abundance of A, B and C in a number of the commoner foods. This table is based upon a similar one compiled by Eddy.

TABLE—SHOWING SOURCES OF VITAMINS

+++ indicates very abundant; ++ indicates abundant; + relatively large; + present in small amount; 0 absent.

Foodstuff	A	B	C
Meats:			
Beef heart.....	+	+	?
Codfish.....	+	+	?
Fish roe.....	+	++	?
Herring.....	++	++	?
Kidney.....	++	++	
Lean muscle.....	0	0	+?
Liver.....	+	+	+?
Thymus (sweetbreads).....	0	0	0
Vegetables:			
Beet root.....	+	+	++
Cabbage, dried.....	+++	+++	+
Cabbage, fresh.....	+++	+++	++++
Carrots.....	+++	+++	++
Cauliflower.....	++	+++	++
Celery.....	?	+++	?
Chard.....	+++	++	?
Lettuce.....	++	++	++++
Onions.....	?	+++	+++
Parsnips.....	++	+++	
Peas (fresh).....	+	++	+++
Potatoes.....	0	+++	++
Potatoes (sweet).....	+++	++	?
Spinach.....	+++	+++	+++
Cereals:			
Barley.....	+	+++	?
Bread (white).....		+?	
Bread (whole meal).....	+	+++	?
Maize, yellow.....	+	+++	?
Maize, white.....	0	+++	?
Oats.....	+	+++	0
Rice, polished.....	0	0	0
Rice (whole grain).....	+	+++	0
Rye.....	+	+++	0
Other seeds:			
Beans, kidney.....		+++	
Beans, navy.....		+++	0
Beans, soy.....	+	+++	0
Cotton seed.....	++	+++	
Peanuts.....	+	++	
Peas (dry).....	+?	++	0

TABLE.—SHOWING SOURCES OF VITAMINS.—*Continued*

Foodstuff	A	B	C
Fruits:			
Apples.....	++	++
Bananas.....	?	+	++
Grapefruit.....	+++	+++
Grape juice.....	+	+
Grapes.....	0	+	+
Lemons.....	+++	++++
Limes.....	++	++
Oranges.....	+++	++++
Pears.....	++	++
Raisins.....	+	+
Tomatoes.....	++	+++	++++
Oils and fats:			
Butter.....	++++	0	0
Cocoanut oil.....	0	0	0
Cod liver oil.....	++++	0	0
Corn oil.....	0	0	0
Cotton seed oil.....	0?	0	0
Fish oils.....	++	0	0
Lard.....	0?	0	0
Oleo, animal.....	+	0	0
Oleo, vegetable.....	0	0	0
Olive oil.....	0	0	0
Nuts:			
Almonds.....	+	+++	
Brazil nut.....	+++	
Chestnut.....	+++	
Cocoanut.....	++	+++	
English walnuts.....	+++	
Filbert.....	+++	
Hickory.....	+	+	+
Pine.....	+	+	+
Dairy products:			
Butter.....	++++	0	0
Cheese.....	++	+	?
Condensed milk.....	++	+	0
Cream.....	+++	+	?
Eggs.....	++++	++	0
Milk powder (skim).....	+	+++	+?
Milk powder (whole).....	+++	+++	+?
Milk whole.....	+++	+++	++
Whey.....	+	+++	+

General Summary of the Nature of Vitamins.—The very small quantities of vitamins that are effective in producing a satisfactory nutritive condition makes them appear distinctly different from foods of the ordinary

nary type. Certainly, vitamins cannot be themselves the sources of much energy in the body; so that they are not utilized to meet fuel requirements, as are carbohydrates and fats or even proteins. The small quantities in which they are effective makes them seem more comparable to such substances as iodine and manganese. But the specific form in which they must be supplied to the body, a form readily destroyed by slight manipulations, indicates that their value is entirely due to some highly unstable atomic arrangement in an organic molecule. Because of the minute quantities of vitamins that occur in nature and their high degree of instability, the chemist is at a serious disadvantage in his efforts to isolate and identify them.

The physiological action of the vitamins may be due to their incorporation into the actual, vital architecture of cells. The close relationship of vitamins to growth processes is perhaps an indication of such a function. On the other hand, the very minute amounts that are effective have been interpreted by some writers as indicating that vitamins function in a catalytic manner. According to this view they would be comparable to iodine of the food. Iodine is utilized by the thyroid gland in the synthesis of a substance called thyroxine. This substance is distributed by the blood and affects oxidations in all parts of the body, in a manner which indicates that thyroxine catalyzes these processes. It remains for future investigation to prove whether or not the vitamins also catalyze certain specific vital reactions.

The common materials of living matter are, then, proteins, fats, lipoids, carbohydrates, inorganic salts, products elaborated from these five groups, and lastly the vitamins; but the vitamins cannot be classified or described chemically; since they are, as yet, known only by their physiological effects.

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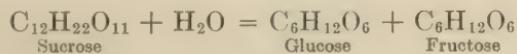
CHAPTER IX

ENZYMES

The chief chemical processes that go on in living organisms may be roughly divided into two main groups: (1) Hydrolysis with its reversed process, dehydration, and (2) oxidation with its reversed process, reduction. Reactions, typical of either of these groups, require powerful chemical reagents or high temperature for their production *in vitro*, that is, without vital activity. Entirely comparable reactions are produced *in vivo* without the presence of powerful reagents or the use of high temperatures. The hydrolysis of cane sugar furnishes a good illustration of this contrast. A solution of the sugar requires 2 N HCl for a given rate of its hydrolysis at 35°C., or a somewhat weaker acid for the same rate at boiling temperature; but the same sugar solution would be hydrolyzed at the same rate or even more rapidly by living yeast cells or by a water extract of the intestine acting without any acid at room temperature. Living mechanisms, then, are remarkably efficient in their power to produce chemical reactions at low temperatures without a lavish expenditure of high chemical energy. The reagents that produce this efficient result are comparable to the catalysts of inorganic chemistry. The catalysts of organisms are called enzymes.

Catalysts.—Catalysts are commonly defined as those substances which affect the velocity of a chemical reaction yet do not take part in it in the ordinary sense, but are themselves unaltered at the end of the reaction. Catalysts are regarded as influencers of chemical reactions. If a substance is to be called a catalyst, its behavior must fulfil certain criteria: (1) It must be effective in quantities that are very small in proportion to the amounts of material that enter into the catalyzed reaction. (2) Since the catalyst itself is not used up during the reaction and can presumably function over and over again, a small amount of the catalyst should produce the same result as a larger amount, provided it is given a longer time in which to act. (3) The catalyst should be recoverable, unchanged, at the end of the reaction, provided no reaction other than the one catalyzed has caused a destruction of the catalyst.

The following are illustrations of inorganic catalytic effects. Hydrochloric acid causes the hydrolysis of cane sugar (sucrose). The real reaction is between sucrose and water, thus:



This same reaction goes on in the absence of hydrochloric acid, but very slowly even in boiling water. The hydrochloric acid merely hastens the reaction and may be recovered unchanged at the end of the experiment. It has not formed a part of the products and has entered into the process only in such manner as to increase the velocity of the reaction. Any acid other than hydrochloric may be used in the same way. All acids have the same effect if present in amounts that furnish the same C_H^+ . This indicates that it is hydrogen ions which really cause the effect on sugar hydrolysis and doubtless they are the main factor; although other factors, such as the concentration of undissociated acid molecules and of inorganic salts also have some effect. But inasmuch as hydrogen ions are recovered unchanged at the end of the experiment, act in very small amounts and otherwise fulfil the above criteria, they are catalysts. Acids, that is hydrogen ions, similarly catalyze the hydrolysis of proteins and many other substances.

Another entirely different type of inorganic catalyst is illustrated in the action of platinum upon the oxidation of hydrogen and other gases. A mixture of hydrogen and oxygen does not appear to effect chemical combination. At least there is no explosion until a spark is applied. As a matter of fact, evidence of an exceedingly slow union to form water molecules has been obtained; but in a reasonable period of time, one year, for example, the water produced is not appreciable in amount. If, however, the mixture of gases is brought into contact with platinum, especially when the platinum is in the finely divided condition called colloidal, hydrogen and oxygen unite rapidly—they burst into flames or even explode. Cigar lighters, in which a mixture of alcohol vapors and air are made to ignite by a platinum covered wire, are a practical application of this principle. The finely divided condition of the platinum is so important in facilitating this effect that the actual amount of platinum surface exposed is apparently the determining factor. The platinum effect on oxidations is therefore interpreted as due to its power to adsorb the molecules of oxygen and oxidizable gas. By the term, adsorb, is meant holding upon the surface and in this particular case, the assumption is that oxygen and hydrogen molecules are held upon the platinum surface in such large numbers that their collisions with each other become effective in causing their chemical union. The platinum is recovered unchanged at the end of the oxidation and may be used over and over. It merely catalyzes the reaction.

Catalysis.—The nature of the action of catalysts is apparently not always the same. The first example of catalysis, that caused by acids, is due to the presence of electrically charged ions. It is an example of many catalytic effects due to the presence of electrolytes. The second example, that caused by colloidal platinum, is due to the adsorbing power of contact surfaces. It is therefore called contact catalysis and is only

one of many similar catalytic effects caused by colloidal substances. Whether or not still other distinctly different types of catalytic effects occur, it is difficult to say; but the two types mentioned, electrolyte effect and contact catalysis, are especially prominent among the catalysts of life processes.

The above statements have assumed that the reaction catalyzed in each case would have gone on without the catalyst but at a much slower rate. This may be true of all catalyzed reactions. Some writers, indeed, go so far as to insist that no substance shall be called a catalyst unless it merely hastens the velocity of a reaction that would proceed without it. This view is perhaps something of a mathematical quibble since it requires that in some cases the reactions proceed at an infinitesimal rate without the catalyst.

When, however, the reaction does proceed without a catalyst at a measurable rate but is distinctly hastened by the presence of the catalyst, the final result may be exactly the same. The reaction proceeds to a certain equilibrium, either with or without the catalyst. The only effect of the catalyst is to cause equilibrium to be attained sooner. The following will serve as an illustration. When ethyl alcohol and acetic acid are mixed in the proportion of one mol of each, they slowly unite to form ethyl acetate and water. This reaction proceeds until about two thirds of the alcohol molecules have combined with an equal number of acid molecules. The reaction then comes to an equilibrium. When suitably catalyzed, this reaction proceeds to the same equilibrium. In either case, the progress of the reaction can be followed by titrating the quantity of free acid remaining in the solution at suitable intervals.

The reaction involved is written thus:



The reaction is reversible and this fact can be shown by mixing one mol of ethyl acetate with one mol of water, after which the reaction reaches the same equilibrium described above. The catalyst likewise hastens this latter or reversed effect. At equilibrium, the reaction is proceeding in both directions but the speed in one direction is so related to that in the opposite direction as to maintain a constant proportion of $\frac{1}{3}$ of a mol of acetic acid to $\frac{2}{3}$ of a mol of ethyl acetate. A similar experiment in which the enzyme, emulsin, from almonds acts upon the hydrolysis and synthesis of a glycerol-glucose glucoside gives results shown in the curves of Fig. 41. The catalyst must act equally upon the two opposing reactions. It affects both hydrolytic and synthetic processes.

All cases of catalytic action are not as simple as those of the preceding examples. Sometimes the reaction appears to go to completion in one direction. Such cases can be explained, however, by the removal of the

products formed. This removal may be due to physical conditions or to a destruction by some subsidiary reaction. When starches, for example, are hydrolyzed in the manner described on p. 108, the dextrin so produced is at once changed to other products so that an equilibrium in the change, starch→dextrin, would not be expected short of a complete transformation. In other cases a catalyst may appear to bring a reaction to

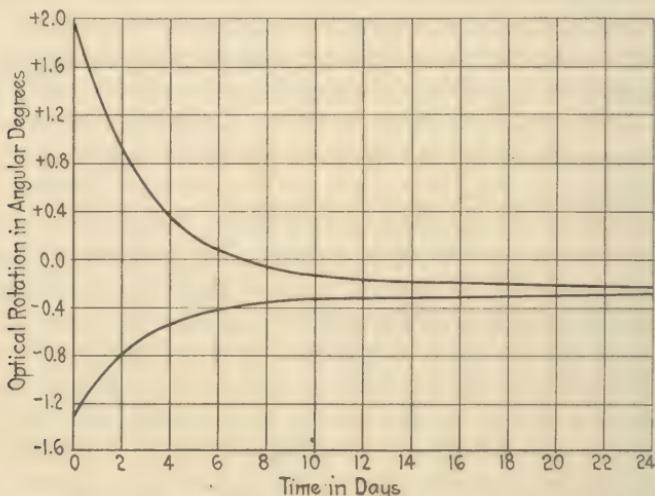


FIG. 41.—Showing that a catalyzed reaction may attain the same equilibrium when proceeding in either direction. The upper curve shows, by the decrease in optical rotation, a diminution of the concentration of glucose present in the original solution. This is due to the synthesis of a glucoside from glucose and glycerol under the influence of the enzyme, emulsin. The lower curve similarly shows the hydrolysis of the same glucoside with a corresponding change in rotatory power under the influence of the same enzyme. The two reaction approach the same equilibrium. (After Bayliss).

a different equilibrium from that attained without the catalyst. Explanations of these cases are also made on the basis of effects of subsidiary reactions.

Enzymes as Catalysts.—Enzyme actions obey the essential criteria for catalysis. This is shown by the study of a specific enzyme, sucrase. This enzyme is obtained from numerous sources, both plant and animal. One preparation of it is made by extracting the lining of the small intestine with water. This enzyme catalyzes the hydrolysis of sucrose. A small quantity of the enzyme solution will effect the hydrolysis of a large quantity of sugar, may be used again and again with successive portions of sugar and does not alter the final equilibrium of the reaction. All other enzymes likewise fulfill the criteria of catalytic action.

One apparent objection to this statement is seen in the fact that enzymes do not continue their work indefinitely. After a sufficient amount of material has been changed by the effect of the enzymes, no further activity is seen. This result is explained, however, by the

destruction of the enzyme in a subsidiary reaction. All enzymes are, to a varying degree, subject to a destructive reaction in the presence of water. This indicates that enzymes are themselves hydrolyzed and thereby lose their catalytic powers. This explanation seems all the more probable since enzymes lose their power more rapidly when dissolved in water, that does not contain substances whose reactions are catalyzed by the enzymes than they do in the absence of such substances. In other words, enzymes last longer if kept at work than if idle. Moreover, comparable destruction of inorganic catalysts have been observed. Colloidal platinum, for example, loses its power to catalyze oxidations after it has come in contact with proteins. Apparently proteins become so adsorbed upon the surfaces of the colloidal platinum that it loses its power to effect contact catalysis.

Another objection to the catalytic nature of enzymes is found in the observation that at sufficiently great dilution of the enzyme its effects can not be detected. In other words, a small quantity of enzyme can not do the work of a larger quantity except within certain limits. This discrepancy can also be explained by a destruction of the enzyme which in this case causes its disappearance before it produces a perceptible effect. On the whole, then, enzymes are to be regarded as catalysts. The specific name, enzyme, is reserved for those catalysts which are produced by living cells.

The enzymes discussed in this chapter are the ones that catalyze hydrolyses. Enzymes, concerned with the other main type of biochemical reactions, oxidations, are just as important as the hydrolyzing ones. But the activity of oxidizing enzymes is so bound up with other vital activity, that their description will be more satisfactorily given in connection with the general subject of physiological oxidation. (Chap. XXI.)

Discovery of Enzymes.—From early times the processes of fermentation were recognized as able to produce marked chemical changes without the addition of chemical reagents. Putrefaction processes, fermentation of fruit juices and many similar processes received considerable attention. During the eighteenth century processes of digestion in animals were shown to involve similar changes. It was eventually shown that whereas the typical fermentations were due to the presence of bacteria, yeasts or fungi, extracts of the stomach could cause protein digestion even when the extract was free from all micro-organisms. In 1814 Kirchoff showed that an extract of barley malt converted starch into sugar and in 1833 the active constituent of the malt extract was precipitated by alcohol, prepared in dry form and shown to have still preserved its starch hydrolyzing power. During the next few decades, extracts of many plant and animal tissues were described as being capable of breaking down proteins, fats or carbohydrates. The reagents, which produced these changes, were all spoken of as "ferments," because their effects resembled those

of the fermenting organisms. To distinguish them, however, from bacteria, yeasts, etc., they were often referred to as the "unorganized ferments."

Although some writers, even at that time, suggested that micro-organisms probably produced their fermenting effects by means of unorganized ferments contained in their bodies, the first attempt to clearly distinguish these active agents was made by Kühne in 1878. He proposed to call these substances, extracted from structures of higher plants and animals and the similar hypothetical agents in micro-organisms, by the name enzymes. This name comes from the Greek, meaning "in yeast" and was intended by Kühne to refer to the fact that such substances produced effects similar to those caused by something in yeast. Other names have been proposed. Of these, "biocatalyst," suggested by Bayliss, has the advantage of being brief and yet indicating the nature of the effects produced by these substances.

The final demonstration of the fact that the fermenting power of micro-organisms is due to the enzymes contained in them, did not occur until 1897. In that year, Büchner showed that ordinary yeast cells yielded a juice that was entirely free from living cells but yet was able to ferment sugar to CO_2 and alcohol very rapidly. His method of preparing the juice was to grind the yeast with fine, sharp sand in order to break up the individual cells and then to subject the resulting mass to very high pressure so as to force out the more liquid portion. A very small amount of this juice would cause alcoholic fermentation of a large quantity of sugar. Since no living matter, that is, no power of growth and reproduction, was present, this effect must have been due to a non-living substance, a catalytic agent. Büchner proposed to call the active agent "zymase." His discovery was a distinct advance in the progress of enzyme chemistry because it showed that a chemical reaction, which supposedly occurred only within living cells, could be produced without the presence of protoplasm.

Intracellular and Extracellular Enzymes.—The only reason why such a discovery was not made earlier was that certain enzymes are held very tenaciously within the cell. Such a retention is in marked contrast to the ready separation of enzymes from their sources as it occurs in the production of digestive enzymes by secretion from glands of the stomach and intestine and in extraction from cells of germinating (malted) seeds. Enzymes of this latter type, those that were early recognized, are easily separated from the structures that produce them and are normally active, indeed, only after they have left their source. Enzymes, like zymase, that normally act within the cell, are termed *intracellular* and are doubtless very numerous. Many have been demonstrated since Büchner's discovery. Those of the other type, which appear in animal and plant secretions, are called *extracellular*.

Recognition of these two types of enzymes has favored the tentative view, sometimes expressed, that all metabolic activities, whether occurring within the cell or outside of it under its influence, are due to bio-catalysts—the enzymes. There is some evidence, however, that protoplasmic activities can, in some cases at least, be carried on without enzymes. The secretion of milk in the mammary gland, for example, proceeds effectively, although no evidence of the presence of any enzymes that act upon the typical milk constituents has so far been obtainable. In spite of these exceptions, the discovery of enzymes and the study of their rôle in cell activities have marked a great advance in knowledge of vital phenomena.

Nomenclature.—A systematic method of naming individual enzymes is desirable because of the large number of different ones that have been recognized. The modern system adds the termination “ase” to the root word referring to the substrate of the enzyme. By substrate is meant the substance that is altered under the influence of the enzyme. Under this system the name sucrase obviously refers to an enzyme that causes the breakdown of the sugar, sucrose. The terms, protease, lipase, glucose, glycogenase, glucosidase, etc. are similar self-explanatory names. Many of the enzymes, such as those of the digestive system, that were studied early in the history of biochemistry, received special names, such as ptyalin, pepsin and trypsin. Each of these names was chosen by an early writer simply in accord with what seemed to him a suitable characterization. Even now that a systematic name is available for each enzyme, the old and well-known names continue to be used. Another system for naming enzymes uses the suffix—lytic. Proteolytic (protein splitting) enzymes, amylolytic (starch splitting) enzymes, are examples of this usage. The inference from such a name is not entirely satisfactory, for just as electrolytic means splitting by an electric force, so proteolytic might mean splitting by a protein. Bayliss has therefore suggested the use of the suffix, -elastie; for example, proteoclastic, lipoclastic. The enzymes are, furthermore, termed hydrolases or oxidases, according to whether they affect hydrolyses or oxidations.

Physical and Chemical Properties of Enzymes. *Solubility.*—The solubilities of enzymes are shown by the methods used in removing them from their plant and animal sources. Most of them can be extracted from suitably ground up tissues with water. After filtering the extract a clear filtrate containing the enzyme is obtained. In some cases it is advisable to dissolve out the enzyme by a salt solution, since certain enzymes appear to be dissolved more readily when the globulins are simultaneously dissolved (p. 37). Many enzymes are best removed from their natural sources by dilute alcohol, though most of them are insoluble in strong alcohol and all are insoluble in absolute alcohol. Glycerol and mixtures of glycerol with water constitute good solvents for enzymes.

Precipitation.—Their precipitation is effected in several ways. As indicated above, they may be precipitated by addition of sufficient alcohol to their watery or dilute alcoholic solutions. They are also thrown down by those metallic salts, such as copper sulphate and mercuric chloride, which precipitate proteins. Indeed, any reagent which precipitates proteins from the enzyme-containing extract of a plant or animal tissue, or from a digestive secretion, also precipitates any enzymes that are present. This fact indicates that enzymes are thrown down by the same agencies which cause precipitation of colloids. Such is indeed the case. Enzymes may be adsorbed upon such colloids as gum mastic and upon subsequent precipitation of the suspended particles of the gum, the enzyme is thrown down with it. This enzyme precipitation may occur even when the precipitating agent used is unable to throw down the enzyme without the previous addition of the gum or other colloid. In other words, the precipitation of any colloid from an enzyme solution is liable to cause the simultaneous precipitation of the enzyme. This is analogous to the well-known mutual precipitation of colloids and suggests very strongly that enzymes are themselves in the colloidal state. In fact all the precipitation behavior of enzymes is typical of the precipitation of emulsoid colloids. This being the case, specific reactions, by which certain enzymes may be thrown down without the simultaneous precipitation of any of another enzyme which might be present in the solution are not known. Enzymes naturally occurring together are not easily separated.

Colloidal Behavior—Further confirmation of the colloidal character of enzymes is found in their behavior toward dialysis. Enzymes do not diffuse through semipermeable membranes such as parchment paper or collodion.

Sensitivity to Temperature.—The behavior of enzymes toward temperature changes is very pronounced though not strictly characteristic of them. The velocity of all chemical reactions is markedly increased by rise in temperature. In a vast majority of cases, the velocity rapidly increases with rise in temperature. This fact is often made use of to distinguish between a true chemical reaction and a typical physical process; since, according to the rule of van't Hoff, the velocity of a chemical reaction is at least doubled by a rise of 10°C., while a physical process is hastened to a less degree by the same rise. Enzymes show the typical effects of temperature upon chemical reactions, but they show these effects only within certain limits of temperature changes. The action of sucrase, for example, in the hydrolysis of cane sugar, though almost imperceptible at 0°C., is markedly hastened by rises in temperature up to about 40°C. This increase in velocity is not continuously proportionate, however, to the rise in temperature, since the effect of a change from 20 to 30°C. is greater than the corresponding effect of a change from

30 to 40°C. Moreover, a change from 40 to 50°C. actually slows down the rate of hydrolysis. At still higher temperatures, the enzyme effect is completely lost and no hydrolysis occurs. For most enzymes a prolonged exposure to a temperature of some 60 to 80°C. or a very brief exposure to boiling temperature permanently destroys the enzyme; so that its activity does not reappear when the solution is again cooled down. The time required for the complete inactivation (destruction) of the enzyme by heat is shortened by rising temperature in such manner as to indicate that the destructive process is a chemical reaction whose velocity is influenced by temperature in the same way as is the characteristic enzyme effect itself. With rising temperature, then, two things are happening in an enzyme preparation: (1) The catalytic effect of the enzyme upon its substrate is being hastened, (2) the reaction which destroys the enzyme is also being hastened. In the lower range of temperatures (below about 50°C.) the first of these effects predominates while at higher temperatures the second is more apparent. So far as the effectiveness of the enzyme is concerned, these two processes are mutually antagonistic. The result is that a rise in temperature up to a certain point hastens the activity of the enzyme, while further increases slow down its effects. The temperature at which an enzyme shows its maximum effect is called its optimum temperature. For animal enzymes the optimum is 38 to 40°C., that is, body temperature. A vegetable protease, papain from the paw-paw tree, has an optimum above 60°C. The marked sensitiveness of enzymes to the destructive effects of high temperatures has often been used as a criterion for the identification of an enzyme. In fact, enzymes are sometimes defined as catalysts produced by living organisms and destroyed in the presence of boiling water. A few exceptions to this rule have been noted, however, in that an enzyme may become reactivated by merely cooling down its hot solution. In any case, the effects of temperature upon different enzymes are not identical and do not serve to characterize the biocatalysts as distinct from inorganic catalysts.

Sensitivity to C_H.—The behavior of enzymes toward changes in hydrogen ion concentration is also very pronounced. Each enzyme is capable of showing its characteristic catalysis only within a certain range of pH values. In about the middle of this favorable range, the velocity of the enzyme effect is at a maximum and at pH values either below or above this optimum, the effectiveness of the enzyme is correspondingly decreased. If an enzyme solution is made either strongly acid or strongly alkaline, its power is completely and irreversibly lost, so that restoration of the solution to the optimum pH of the enzyme does not restore enzyme activity. Different enzymes vary widely as to their optimum pH. Pepsin, the protease of gastric juice, is active in the range 1-3 of pH values with Yan optimum at approximately 1.8-2; while trypsin, the

protease of pancreatic juice, is active in the range 6.8–9 of pH values with an optimum at about 8.2. Gastric juice is normally acid with a pH near to that which is optimum for pepsin, while pancreatic juice is alkaline and similarly corresponds in pH to the optimum of trypsin. If either of these juices is changed to the reaction of the other, its protease is destroyed. These two enzymes mark the extremes of the optimal hydrogen ion concentration for enzyme activities. The majority of enzymes have optimal hydrogen ion concentrations closer to neutrality. All are, however, greatly changed in effectiveness by slight variations from the optimum and are destroyed by greater variations. One of the reasons why living matter is very sensitive to changes in C_H is doubtless this effect upon its catalysts.

Behavior to Antiseptics.—In one respect, however, enzymes are sharply differentiated from living matter. They show their characteristic effects in spite of exposure to antiseptics that destroy life. Chloroform, alcohol, ether, sodium fluoride or any one of a long list of antiseptics may be present in an enzyme solution, in concentration that would rapidly kill all living matter, and still cause no interference with the activity of the enzyme. This fact further emphasizes the above statement that enzymes are non-living agents, entirely distinct from protoplasm, even though they are always produced by living cells and sometimes appear to act only within the protoplasm. The use of antiseptics in a solution to be tested for enzyme action is an important aid in distinguishing the enzyme effect from that due to any bacteria or other micro-organisms which might accidentally get into the solution and so multiply, in the absence of antiseptics, that their metabolism would simulate or conflict with the enzyme effect.

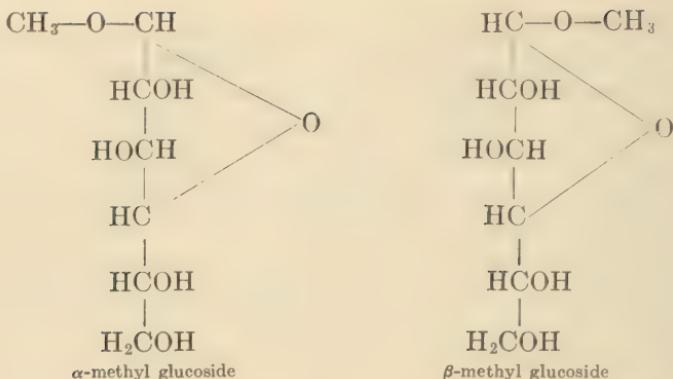
Not all substances used as antiseptics permit satisfactory enzyme action in their presence. Certain metallic salts, as for example, mercuric chloride, and all substances that produce an unfavorable C_H of the solution inhibit or destroy enzyme activity. Such an effect is analogous to the so-called "poisoning" of inorganic catalysts.

The Chemical Nature of Enzymes.—Extensive and numerous researches to determine the chemical composition of enzymes, though revealing many significant facts, have not yet arrived at any definite and final conclusions. By the methods of dissolving and precipitating mentioned above, concentrated preparations of various enzymes have been made. Whether any of them consisted of an isolated enzyme, entirely free from other material, is quite impossible to say, because there is no criterion of enzyme purity. Moreover, many of the methods that would ordinarily be available for the separation and purification of most substances cannot be used in enzyme preparation without causing the disappearance of enzymatic powers. Enzymes cannot be subjected to any processes of heating, cannot be crystallized or distilled. Another

difficulty arises from the fact that enzymes are not, in a certain sense, unit substances at all. Their appearance and disappearance, in accord with changes in hydrogen ion concentration, is alone an indication that enzymes do not exist as such except under certain conditions. A clearer proof is found in experiments on the effects of dialysis of enzymes. Solutions of certain enzymes, dialyzed against distilled water, lose their power, although the enzyme can not be found on the water side of the dialyzing membrane. Something requisite for enzyme action diffuses through the membrane. These diffusible substances are electrolytes. If the diffusible material is restored to the solution containing the colloidal (non-diffusible) material, the enzymic power is regained. This indicates that the *enzyme activity, in some cases at least, is really the result of the interaction of at least two constituents, one of which is a colloid and the other a crystalloid.* The colloidal constituent, after being inactivated by dialysis, can be restored to activity by the addition of chlorides or bromides in some cases or of phosphates or nitrates in others. The hydrogen ion concentration must also be correctly adjusted. The chemical nature of the colloidal constituent is probably that of a protein in the case of pepsin and trypsin. At any rate, the purest and most powerful preparations of these enzymes give the typical protein tests. The colloidal constituent of sucrase, on the other hand, appears to be a carbohydrate though different investigators do not agree as to whether or not it is entirely free from protein. Perhaps the best that may be said, at the present stage of research in enzyme chemistry, is that the colloidal material of different enzymes is diverse in chemical structure.

Specificity of Enzymes.—The diversity of the composition of enzymes is further shown by the specific nature of their effects. Indeed, enzymes are only recognized as distinct from each other because they catalyze different reactions. They vary, however, as to the degree of their specificity. Some are specific only in the sense that they catalyze one large group of reactions. For example, most proteases catalyze the hydrolysis of practically all proteins. Other enzymes are slightly more specific. For example, the amylases of animal digestive juices cause the hydrolysis of starches and glycogen but have no effect on other polysaccharides even though the latter are hydrolyzed by inorganic catalysts, such as hydrochloric acid, just as readily as are starch and glycogen. Some enzymes are so highly specific that they affect the hydrolysis of one and apparently only one substance. For example, lactase of the intestine appears to be specific in its action upon lactose.

The specificity of enzymes is peculiarly related to the stereoisomerism of the substrate. One of the clearest examples of this relationship is found in the action of enzymes upon certain glucosides. The following formulas of α - and β -methyl glucoside show that the two substances differ only in the stereo arrangement of the methyl group. Yet they show



marked contrast in their susceptibilities to enzyme effects. The α compound is split by maltase from any source, either animal or vegetable; while the β variety is not attacked by any maltase but is readily split by an enzyme, called emulsin, that is found in peach stones, almonds and certain other vegetable structures. Emulsin does not attack the α -glucoside.

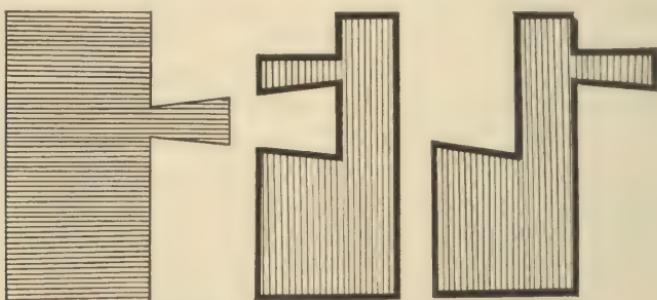


FIG. 42.—Diagram to suggest the lock-and-key theory.

These facts and similar ones lead to the "lock-and-key" theory of Fischer. According to this theory, the enzyme has such a surface shape of its molecule that it fits upon the molecular structure of the substrate, somewhat as the shape of a key enables it to fit a particular lock. The above diagram will serve to illustrate the meaning of the theory.

A large group of compounds that have a structural arrangement similar to that of α -methyl glucoside are all acted upon by maltase, while another large group, having the β structure, are all broken down by emulsin. This fact shows that enzymes are not specific for certain individual substrates, but are specific for certain atomic groupings which constitute merely a portion of a molecule. In accordance with the modern theory of the electronic structure of matter, the colloidal particles of an enzyme might be regarded as having a certain arrangement of electrical charges which is adapted to attract to itself another specific

arrangement, or "pattern," of charges but not other "patterns" that differ in certain particulars from the one to which it is adapted. The ability of colloids, other than enzyme constituents, to show a so-called specific adsorption, has been recognized. Filter paper, for example, adsorbs potassium more readily than calcium and gelatin is stained by the adsorption of certain dyes much more readily than by that of other dyes. The specificity of enzymes is attributed, then, to the specific adsorbing powers of the colloidal material which is an essential component of each of the combinations of things that we call enzymes.

The Nature of Enzyme Action.—The first step in the process of enzyme catalysis is apparently a combination between the enzyme and its substrate. Evidence for this statement has been obtained in several lines of experiment. For one thing, the protective action of the substrate for the enzyme has been observed, as mentioned above. An enzyme is not so readily destroyed, when in water solution, if an abundance of its substrate is present. Pepsin, for example, in a solution rich in proteins, will not disappear in the course of a week at room temperature but dissolved in water without proteins the pepsin disappears within a day or two. The protective action of the substrate is exerted in spite of the fact that it is itself simultaneously broken down. This merely indicates that as fast as the adsorbed substrate is destroyed, other molecules of the same substrate are adsorbed upon the enzyme to take its place.

Another proof of combination between enzyme and substrate is found in the study of the effects of varying the concentration of the substrate. In a series of tests having the same concentration of sucrase but having different concentrations of sucrose in each test, it is found that the velocity of sucrose hydrolysis increases with increasing concentration of sucrose up to a certain concentration. These variations in velocity are found to be in accord with the law of mass action as it relates to the velocity of a simple, chemical reaction. Greater concentration of the substrate, beyond this critical value, has no further effect in increasing the velocity of hydrolysis. This means that when the substrate is so abundant in the solution that it is able to keep all of the enzyme saturated, as it were, by replacing with new molecules of the substrate all those that are hydrolyzed, no further increases of substrate concentration can hasten the process. In other words, when all the enzyme is forced to combine with substrate, the enzyme will do its maximum work.

After the enzyme-substrate combination has occurred the subsequent events probably depend upon the action of ions. Hydrogen and hydroxyl ions are doubtless effective in all cases, since variations in their concentration markedly alter the activity of any enzyme. In certain types of hydrolyses, they may be the only ions concerned. This seems to be the

case in the action of pepsin, because a given concentration of hydrogen ions and a consequent fixed concentration of hydroxyl ions produces the same rate of activity of a given pepsin preparation even though any one of a large number of different acids is used to furnish the desired C_H. Whether or not hydrogen and hydroxyl ions are themselves adsorbed upon the surfaces of the colloidal particles of the enzyme, thus rendering the ions more effective hydrolytically, is not certain, but seems probable judging from analogy with inorganic contact catalyses. In other types of hydrolysis, specific ions, other than H' and OH', are effective. The Mn'' ion is very effective for the activity of certain plant enzymes, while the ions furnished by dilute phosphoric acid are especially effective in furthering the activity of glycogenase of liver and other animal tissues, although Cl' and certain other anions are also effective to a lesser degree. The effectiveness of H' and OH' ions in these reactions is readily understood from the very nature of hydrolysis (p. 21), but the usefulness of other ions remains to be explained. It is still more difficult to conceive of an explanation of the usefulness of ions in those enzyme effects that produce oxidation instead of hydrolysis. The nature of oxidase action will be considered later.

Another peculiarity of enzyme action is its ability to occur when the enzyme is not in solution. Sucrase, for example, has been shown to be able to hydrolyze cane sugar when the enzyme lies in precipitated form under a solution containing a concentration of alcohol in which the enzyme is entirely insoluble. This fact is not peculiar, however, to enzyme catalysis. Platinum black and other colloidal materials, that effect contact catalysis, act without being themselves in solution. It is true, however, of any colloidal catalyst that the more highly it is dispersed, that is, the smaller its particles and the more perfectly they are distributed throughout the reacting mixture, the more rapid is the catalytic effect. This is due to the fact that greater dispersion permits exposure of a decidedly larger adsorbing surface. When a spherical particle is divided into smaller ones, whose radius is $\frac{1}{1,000}$ of the original sphere, the total surface of these smaller particles is a thousand times that of the original particle.

Activation of Enzymes.—Another peculiarity of certain enzymes is that they need to be activated in some manner. Several different extracellular enzymes are known to be produced by their secreting cells in an inactive form, called *zymogen*. This form is regarded as the precursor or forerunner of the enzyme, since it must be brought into contact with some activating agent before it acquires enzymic powers. Pepsin, for example, when obtained by extracting gastric glands, is not proteolytic, but becomes active only after exposure to acidity. In the stomach, this activation is normally produced by the hydrochloric acid

secreted by certain specialized cells in the gastric mucosa. The zymogen of pepsin is called *pepsinogen*. It is not merely pepsin minus the hydrogen ions that are required for its activity. It is distinctly different from pepsin, because it can be exposed to a certain degree of alkalinity and still show characteristic activity when brought back to the optimum pH (1.8) while a similar exposure of pepsin to the same alkalinity would permanently and irreversibly destroy it. In other words, pepsin, once activated by exposure to the correct CH^- , is more sensitive to the destructive effects of alkali than is pepsinogen. Another example of activation is found in the case of trypsin of pancreatic juice. If pure pancreatic juice is obtained from the pancreatic duct in such manner as to be entirely uncontaminated by other body fluids, it is found to possess no proteolytic power. But if this juice comes in contact with even a mere trace of intestinal secretions, it rapidly develops tryptic activity. Since a small quantity of intestinal juice will cause this activation as completely as will a larger quantity, but will do it at a slower rate, the activation is a catalytic process. In other words, it is due to an enzyme. It was named *enterokinase* by its discoverers, Bayliss and Starling. We have, then, this peculiar case of an enzyme that produces an enzyme. Other instances of the production of zymogens and their subsequent activation have been described but the two given above, the one activated by ions, the other by an enzyme, are the most thoroughly investigated cases and serve to typify such reactions.

Co-enzymes.—Even after an enzyme is suitably activated, it requires the presence in its immediate vicinity of certain ions or possibly of certain undissociated substances in order to do its work. The need of certain ions was described above. The requisite substance, other than the colloidal constituent of the enzyme, is spoken of by some writers as a co-enzyme. This word is ill-defined and unfortunate in its implications since it suggests that ions and other known substances that further enzyme effects are themselves enzymes. It seems better to regard an enzyme as a combination of things: A colloidal, specifically adsorbing substance, and another substance or group of substances which cooperate with the colloidal constituent to produce catalysis. In addition to the several positive and negative ions that have been recognized as specifically influencing certain enzyme effects, certain compounds peculiar to the bile have been mentioned as co-enzymes for pancreatic lipase. But since these compounds, as explained in Chap. III, have remarkable influence on surface tension and therefore upon the colloidal dispersion of fats, there is some question as to whether bile really enters into the catalytic effects of lipase or merely renders the substrate, fat, more accessible to the enzyme.

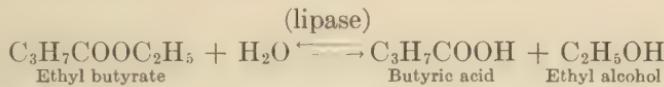
Anti-enzymes.—The term anti-enzyme is used to refer to substances which inhibit the action of enzymes. The word was coined in the belief

that such substances were immune bodies like antitoxins. It was believed that an animal injected with an enzyme, foreign to its body, developed a substance specifically counteracting the enzyme injected. As certain of the earlier experiments have been thrown into doubt by later investigations, the use of the word anti-enzyme with the implication that it refers to an immune substance, is not entirely justified. One clear instance of the occurrence of an enzyme inhibitor is found in the case of tape-worms. These animals live as intestinal parasites in the presence of the powerful pancreatic protease, trypsin, but are not themselves digested by it. Water extracts of dead tape-worms prevent the action of trypsin when mixed with pancreatic juice. The nature of the inhibiting substance is unknown. A tape-worm extract can only inhibit trypsin during a limited time, so that the substance is merely an inhibitor and does not destroy the enzyme.

Anti-enzymes have been postulated as existing in the lining of the stomach and intestines so as to protect them from the powerful enzymes of the digestive juices. Direct experimental evidence of such inhibitors is lacking. It seems more probable that the stomach and intestine do not digest themselves, because food substances are present in sufficient amounts to cause nearly complete combination with the enzymes present. The lining cells are further protected from the action of proteases by an abundant mucus secretion which forms a slimy coating over them and furnishes enough of the protein, mucin, to combine with any protease not otherwise engaged. Many kinds of living cells, bacteria, yeasts, and molds, for example, resist the action of enzymes, even though their constituents may be easily acted on when the cells are dead. This indicates that living cells are not very permeable to enzymes. In spite of that, the cells lining the intestine are markedly irritated, presumably, by processes analogous to digestion, if pancreatic juice is secreted into the intestine under artificial stimuli applied when the intestine is empty. Similar irritations occur in the lining of the stomach during hypersecretion of gastric juice, that is, when gastric juice is secreted in excess of that required for digestion of the food. These facts indicate that, in the absence of sufficient food material to furnish substances adequate for combination with the digestive enzymes, the latter can, to some extent, attack the cell surfaces of the digestive mucosa.

Reversibility of Enzyme Effects.—All typical catalysts produce the same final equilibrium in the reactions which they affect, as would be obtained in the absence of the catalyst. In the illustration given above of the action of HCl upon the hydrolysis of ethyl acetate, the catalytic effect is also exerted equally well, upon the synthesis of ethyl acetate from its hydrolytic products, alcohol and acetic acid. The catalytic effect is said to be reversible. Enzymes as catalysts also show reversible effects. The first clear demonstration of this fact (1900) was obtained in investiga-

tions on pancreatic lipase. A water extract of the pancreas, treated with a small quantity of butyric acid and ethyl alcohol, quickly develops the unmistakable odor of ethyl butyrate (essence of apples). Kastle and Lovenhart, who discovered this reaction, showed that it occurred according to the laws of enzyme action and did not take place if the pancreatic extract was first boiled. They also showed that the ethyl butyrate thus produced could be distilled off and, when added to fresh pancreatic extract, be hydrolyzed into butyric acid and alcohol by the same enzyme which had produced it. This hydrolysis is detectable by titrations which show the quantity of free butyric acid present at any time during the progress of the reaction. Such observations demonstrate that the reaction merely comes to an equilibrium at which a given concentration of the ester (ethyl butyrate) is balanced by a given concentration of butyric acid and ethyl alcohol. This does not mean that reaction ceases when equilibrium is attained. It merely signifies that, as in all chemical equilibria, the masses (effective concentrations) of the several substances concerned in the reversible reaction are so adjusted that the rate of reaction in one direction is just equal in the reverse direction. From this point of view, the reaction of lipase upon ethyl butyrate should be represented as a reversible reaction, thus:



A similar effect of pancreatic lipase in the production of true fats, such as the glyceride, triolein, has also been demonstrated. Fats are synthesized, then, as well as hydrolyzed, by the same enzyme.

Attempts to demonstrate the reversibility of enzymes acting upon carbohydrates, in other words, to show enzyme synthesis of polysaccharides, have lead to confusing results. The work of Hill (1898), involving the treatment of a concentrated glucose solution with yeast maltase, demonstrated the production of a disaccharide, which he supposed was maltose. But further investigation proved that his synthetic product was really isomaltose. This result cast doubt upon the true reversibility of the enzyme, since isomaltose is not hydrolyzed by maltase. Still further confusion arose when Armstrong showed that glucose solutions acted on by emulsin preparations, such as are made from almonds, yielded maltose. Now emulsin is an enzyme which hydrolyzes all β -glucosides, including isomaltose which has a β -glucoside structure, but does not hydrolyze α -glucosides such as maltose. Both maltase and emulsin, then, appear to reciprocate with each other without being actually reversible. One plausible explanation of this curious anomaly is that maltose preparations contain some emulsin while emulsin preparations likewise contain a little maltase. In one case, at least, such a

natural mixture of these enzymes has been proved to occur and, until further evidence is obtained, the results can best be interpreted as in accord with the idea of a true reversibility of enzymes.

The reversibility of a protein-splitting enzyme has also been demonstrated by Taylor. A concentrated solution of the amino-acids, derived from digestion of the protein called salmine, was treated with a glycerol extract of pancreas, containing active trypsin. After five months a small quantity of protein was obtained. This protein gave all the reactions of salmine.

Although the above cases of reversible action demonstrate this property for a number of representative enzymes it is nevertheless true that reversibility has not been proved for all enzymes. This fact does not preclude a belief in the reversibility of enzymes, however, because many circumstances enter into the catalyzed biochemical reactions in such manner as to prevent reversion. (1) The removal of the products of reaction from the sphere of influence of the enzyme is one such factor. During digestion, for example, absorption of the digestive products into the walls of the intestine prevents reversion. In many cases the progressive steps of enzyme hydrolysis cause further disintegration of the products of each chemical reaction as rapidly as they are formed. The successive steps in the hydrolysis of starch to glucose furnish an example of this type of interference with reversibility. (2) The large proportions of water present in the reacting mixtures tend to prevent reversibility of the action of any hydrolyzing enzyme. This is in accord with the law of mass action since water is a product of any hydrolysis. A specific demonstration of this effect of water was shown in the case of the enzyme lipase acting upon the synthesis of oleic acid and glycerole to form the fat, olein. These experiments showed that the greater the proportions of water present, the less was the synthesis of fat. A larger proportion of water tended to force hydrolysis nearly to completion. A mixture of a given proportion of water, glycerol and oleic acid comes to the same equilibrium under the influence of lipase as does a mixture of corresponding proportions of fat and water but the equilibrium is shifted towards hydrolysis by excess of water or towards synthesis by a scarcity of water. The same effects may be produced by varying the proportions of other constituents so that equilibrium is simply in accord with the law of mass action. These facts are shown in the curves of Fig. 43.

Physiological Significance of Reversibility.—The significance of the reversibility of enzyme action lies in its ability to explain many of the facts of metabolism. Anabolism and catabolism are in many cases reverse chemical reactions. For example, the building up of storage glycogen from sugar is anabolic, while the breaking down of glycogen to form transportable sugar is catabolic. These two processes may well be due to the action of one enzyme and the circumstances, which determine

whether a building up or breaking down shall occur, are those conditions capable of shifting the equilibrium of this reversible, catalyzed reaction. Among such circumstances are concentration of glycogen and of sugar and, especially, concentration of water in that particular portion of any cell, in which glycogen deposits are formed or broken down. The reversibility of lipase action, described above, similarly applies to the explanation of fat metabolism. Fats, like carbohydrates, are stored in a form

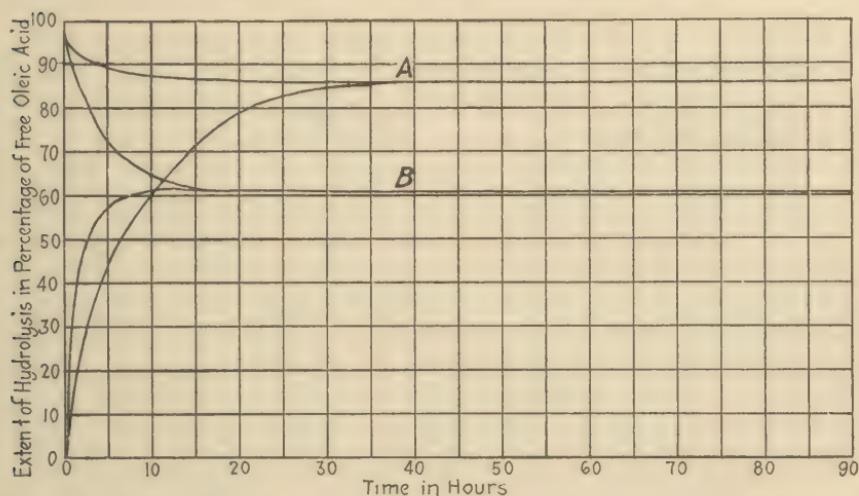


FIG. 43.—Showing that the concentration of water influences the reaction equilibrium attained under the action of a hydrolyzing enzyme. The pair of curves which show attainment of equilibrium at *A* represent the synthesis (upper curve of pair) and hydrolysis (lower curve) of fat under the influence of the enzyme, lipase, of the pancreas when the reacting mixture contains 9 mols of water to 1 of fat. The pair of curves shown at *B* represent the corresponding results when the reacting mixture contains 3 mols of water to 1 of fat. With the smaller concentration of water, hydrolysis, as measured by the free fatty acid present, is less complete and synthesis more pronounced when equilibrium is reached. (After Armstrong and Gosney.)

different from that in which they are transported in the blood. Fats are stored as neutral fat and transported largely, if not exclusively, as lipoids. This fact implies that tissue lipase causes fat synthesis preparatory to storage and fat hydrolysis preparatory to the production of lipoids that are then transported to the seat of active metabolism. Many metabolic reactions have not as yet been definitely shown to be due to enzymes but inasmuch as all reactions are probably reversible under the right circumstances, we find the idea of shifting equilibrium an explanation of one aspect of metabolism: Its power both to build up protoplasmic materials from its foods and to subsequently break them down again. The metabolic reactions that actually furnish energy are oxidative rather than hydrolytic and proceed under such circumstances that many of the reactions involved are not reversible.

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CHAPTER X

THE PHYSICO-CHEMICAL STRUCTURE OF LIVING MATTER

In the preceding chapter, the reagents of living matter, enzymes, were shown to confer a high degree of chemical efficiency upon life processes, so that profound chemical changes occur without the use of high temperatures or highly reactive reagents such as are employed in inorganic chemistry. But properties of living matter, other than the presence of enzymes, also give it a peculiar chemical efficiency. These properties are due to structure, in other words, to the manner in which the components of living matter are put together. The chemical components are probably not different from substances producible by non-living agencies. Many of them, indeed, have been artificially synthesized, as explained in preceding chapters. But even if all the different proteins, fats, carbohydrates and other substances of living structures could be artificially synthesized, it would still be impossible, in the light of our present knowledge, to put these substances together in such manner as to produce life. The architecture of living matter is exceedingly complex.

Moreover, the natural organization of living matter, occurring as a result of growth and development, cannot be changed without profound alteration of vital phenomena and probably death. The generally fatal effect of crushing and cutting living matter is perfectly apparent, but even slight changes in physical structure may produce profound changes in behavior. For example, slight puncture of the membrane that surrounds an egg cell markedly alters its rate of oxidation and is very apt to be fatal. The structure of any given cell very specifically determines its behavior.

Living Matter a Solution.—Despite the complexity of organization of living matter, many facts relating to its make-up have been disclosed. Of these, an outstanding feature is the fact that protoplasm is a solution. The solution is of differing consistency in the different parts of cells. It is almost as fluid as water in some parts and of a highly viscous nature or even a fairly stiff gel consistency in others. Moreover, the protoplasm of a given part of the cell may alter its consistency, changing reversibly from a more fluid form to a comparatively gelatinous condition. This happens, for example, during electrical stimulation of certain cells and during certain stages of cell division of echinoderm eggs. But as gels, even those of stiff consistency, are really forms of solution, the general statement that protoplasm is a solution holds true.

One clear demonstration of this fluidity is seen in the case of certain mycetozoa. These organisms are naked protoplasmic masses that are found on dead wood. Even macroscopic examination enables one to see that they are fluid, since they slowly move by streaming of their protoplasm. This stream motion is still more apparent under magnification. But the most remarkable demonstration of their liquid consistency is seen in their filtration through cotton. They "creep" through the cotton in fine streams which run together again and form organisms having

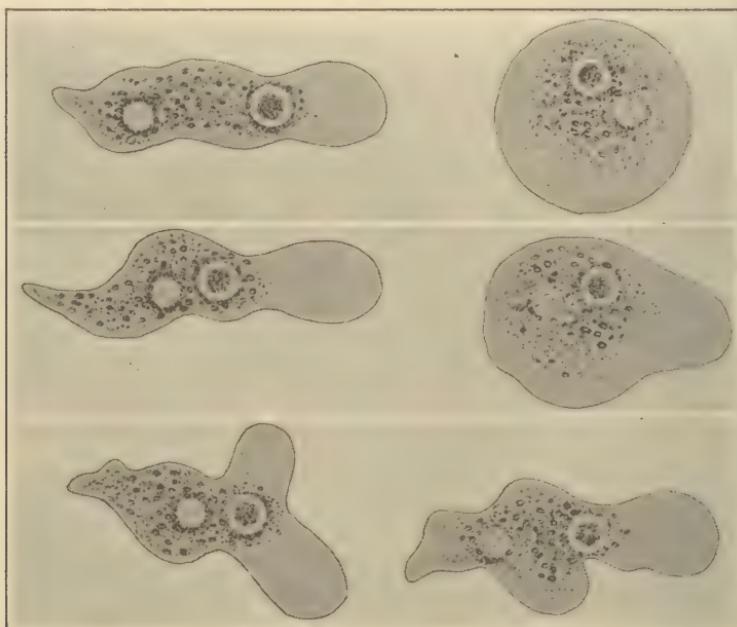


FIG. 44.—Forms taken by an amoeba as the result of amoeboid motion.

the same behavior that they had before filtration. The protoplasm must be finely divided while passing through the cotton because small solid particles, food material that had previously been taken into the protoplasm, are removed by the cotton. The filtered protoplasm is clear. The filtration process can be applied to this form because it is peculiarly devoid of any fixed limiting membranes.

Protoplasmic streaming, however, can be seen in organisms that possess more definite limiting membranes. The amoeba and all structures, such as leucocytes, that show amoeboid motion (Fig. 44) demonstrate the fluidity of living matter. Projections, called pseudopodia, are formed by protoplasmic flowing and into the projection the protoplasmic material may continue to flow so as to cause the entire organism to move along toward food masses which are finally enclosed by pseudopodia and taken into the body of the cell. Even in plant cells, having definite

form with a fixed cellulose wall, the protoplasm within the cell is fluid and motile. Plant cells that occur in isolated form so that they may be conveniently observed show protoplasmic streaming under the microscope. *Tradescantia* hairs are often used for this demonstration. (Fig. 45.)

Even tissues so solid as those of muscles have partly fluid contents in their cells. It is perhaps difficult to think of muscle substance as a solu-



FIG. 45.—A *Tradescantia* hair cell. The granular threads show protoplasmic streaming under the microscope.

tion because muscle appears to be organized in a very permanent and complex form and, indeed, its component cells do possess definite external walls and elaborate semi-solid internal structures. But the mere fact that a muscle changes its shape during contraction is proof of the plastic nature of its component cells. Moreover, Kühne has reported an observation in which a parasitic worm was found inside a living cell of frog muscle. The worm was seen to swim through the protoplasm, thrusting aside the visible structural elements, the fibrils with their cross striation bands, as it swam. The striations fell back into place again as the worm moved on. At one place, however, where the muscle cell had been injured so that its protoplasm was dead, the worm could not penetrate. This observation indicates that living matter loses its fluidity at death, becoming at least semi-solid, and is in accord with the phenomenon of *rigor mortis*, the stiffening of muscles that occurs shortly after death. But living muscles are composed of cells within whose elastic walls is a highly fluid substance surrounding the semi-solid structures, such as fibrils.

Similar statements may be made concerning all living tissues, for although the component cells always contain some small particles and definite structures of apparently solid or semi-solid consistency, yet the protoplasm is partly made up of fluid material, a solution. This fluid part usually appears homogeneous to microscopic observation.

Clear specimens of protoplasm, those that do not contain visible particles, permit the free diffusion of dyes until the dye is seen to be uniformly distributed just as it would be in an artificial solution.

Protoplasm also possesses surface tension such as solutions commonly show. This fact is demonstrable in several ways. For example, vacuoles in the substance of an amoeba tend to be spherical in shape according to the principle of least or minimal surface (p. 78). Also, when proto-

plasm undergoes plasmolysis it tends to draw into spherical droplets according to the laws of surface tension of solutions.

Intercellular Substance.—Accepting the idea that all living matter, with the exception of its membranes and other visibly semi-solid structures, is a solution, one must consider the hard, dense fibers and woody structures of plants and the bones, tendons, cartilage, etc. of animals as composed largely of non-living substance. Microscopic study of such structures confirms this idea; for all hard tissues are found to contain only a few living cells having typical fluid protoplasm within them, but

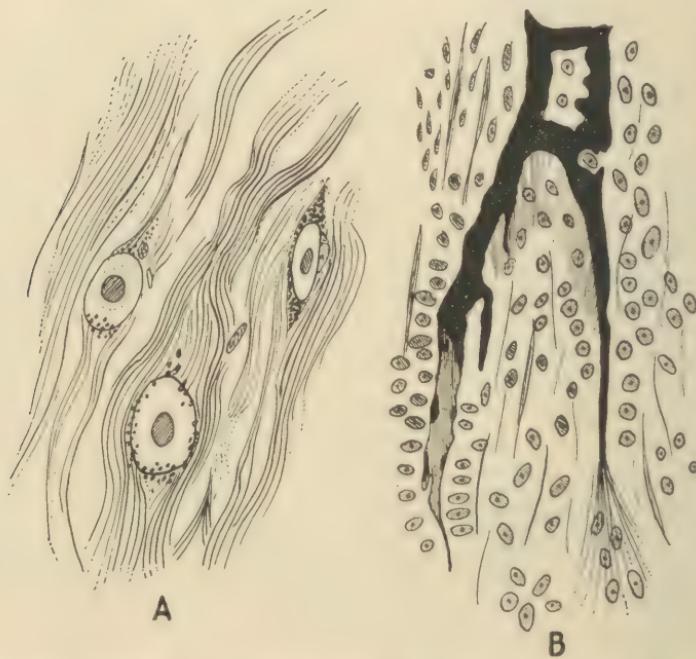


FIG. 46.—To show the comparatively large proportion of intercellular substance in certain tissues. *A*, tendon. *B*, cartilaginous tissue. (After Lewis and Stohr "Textbook of Histology.")

contain much non-living hard material that is really intercellular. This intercellular material can grow or regenerate after injury only in so far as the living cells produce it. The large proportion of intercellular material in certain hard tissues is shown in Fig. 43.

The Polyphase Character of Protoplasmic Solutions.—Although the true living matter is fluid, it is not a simple or even homogeneous solution. Many of its constituents are typical colloids, proteins, polysaccharides, fats and lipoids; so that protoplasm is a colloidal solution. But it is not even so simple as are typical colloidal solutions, because it is not composed of merely two phases, the dispersed and the continuous. It is polyphase in that it contains colloidal solutions within colloidal solutions,

Portions of protoplasm are of a gel consistency, others of the emulsion type and still others are emulsoid. The different types are enclosed, in some cases, the one within the other and various portions of a living cell are furthermore separated from each other by membranes which may be either permanent throughout the life of the cell or may be temporarily produced. Crystalline structures of fibrous or other forms occur in cells either temporarily or permanently. The fact that living matter is fluid, then, does not simplify physiology, because the solutions that we call protoplasm are so complex.

The Cellular Organization of Living Matter.—The general concept of the organization of all living matter into cells has become a commonplace of biology. It is also clearly recognized that even in large organisms the component cells are, as a rule, of very small dimensions. In most cases, cells are microscopic. The few exceptions include the egg cells of many forms, such as fishes, amphibians and invertebrates. Eggs of birds are not a real exception to the general rule; for, although comparatively large, such eggs are really composed chiefly of non-living matter, non-living in the sense that it is incapable of growth or of typical protoplasmic, oxidative metabolism. Only a microscopic portion of even the largest avian egg is really protoplasmic and this constitutes a simple cell. One other notable exception to the rule of small size of cells is seen in the case of muscle cells. Certain of these are spindles, several centimeters in length. But even these cells have a small cross-section of which the diameter is usually microscopic and is always less than 0.1 mm. Although a few other exceptions might be mentioned, it is nevertheless true that protoplasm is characteristically organized into minute cells.

The forms of cells are almost infinite in variety. A cell that grows in an isolated position tends to be roughly spherical; but very few cells retain this shape, because internal forces and external pressures usually modify it. Massed groups of cells as found, for example, in the liver or in the tubers of plants, are irregularly polyhedral in shape as any soft masses pressing upon each other would be. Many cells attain highly specialized forms that are developed during the embryological stage or the growth processes of plants or animals. Some extreme examples of such specialization are the slender, fiber-like cells of skeletal muscle, the irregular shaped nerve cells with numerous nerve fiber processes which in some cases are a meter or more in length, and the cylindrical cells of the hair-like processes of certain plants. Some cells are highly plastic and, as they lack a fixed cell wall, are able, amoeba-like, to change their shape almost continuously.

The forces that determine the form of cells are largely within the cell itself. The physico-chemical constitution of the cellular material at the time the cell arises acts as a pre-determined influence which insures that

the cell shall approximate to a certain type, if it develops at all. Some of the conditions which determine form must be imposed upon each cell by its parent cell, for all available evidence clearly indicates that every living cell originates from a preexisting one. This means that many of the factors (materials and their organized arrangement) which determine the form of each specialized cell of a human body are present in the fertilized ovum and that as each stage of embryonic development occurs, these factors are so segregated by the successive cell divisions that an orderly process of well-defined form-development is assured. Only to a limited degree can external forces alter the character of this development. For example, abnormal pressures, abnormal nutritive supplies or abnormal fluctuations in temperature during embryonic life may produce monstrosities such as headless embryos, Siamese twins, etc., but no known processes can cause a cell, when it is differentiated so as to be the immediate precursor of liver cells, to develop into muscle cells or anything but liver cells.

The Significance of Cellular Structure.—The small size and definitely predetermined form of cells make possible the organization of living matter for specific physiological behavior. This organization of many small units has certain advantages over larger undivided masses. An increased efficiency and intensity of physiological processes is thereby secured. For a rough analogy one may take a battery of galvanic cells. Twenty cells of one volt e.m.f. may be connected in series to produce a current of twenty volts, but a similar large cell of twenty times the capacity of the smaller ones can give only one volt. Although the e.m.f. of a battery is not closely analogous to the forces concerned in most physiological processes, it aptly illustrates those prevailing in the shock given by the electric organs of torpedo rays and certain other electric fishes. These organs can deliver a shock of 200 to 300 volts when the fish is stimulated and are able to produce this high intensity of e.m.f. because of their organization of innumerable units.

Another advantage of subdivision is the increase of surface tension thus obtained. If a spherical mass is divided into 1,000,000 equal spheres the total surface is increased 100 times. In addition to the increase in surface, the tension upon the surface of each of the small spheres is greater than on the larger one because of the greater curvature of the small spheres. Surface tension plays a considerable rôle in all cell phenomena and the minuteness of the organization of living matter makes this force of a sufficient intensity to be effective.

A third advantage of cellular structure is the rapidity of diffusion of substances into living matter and out of it, that is thus made possible. All of the thousands of cells composing an organ of a plant or animal are in intimate contact with the surrounding nutrient medium, blood, lymph or plant juice. Since each cell is very small, efficient and rapid exchange between the cell and its environment is possible.

There are other advantages of cellular organization, but these are sufficient to emphasize its physiological significance. It has been generally accepted as a biological theorem that living matter can exist in an active state only when organized in the cellular form, and that each cell is a unit—is in a certain sense, an individual organism.

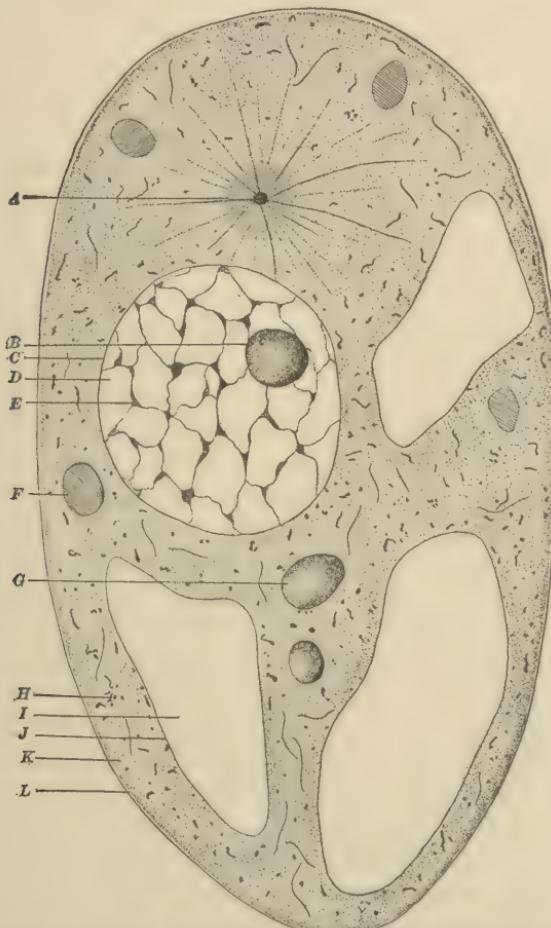


FIG. 47.—Diagram of the cell, showing its principal constituent parts. *A*, centrosphere with centrosome and aster. *B*, nucleolus. *C*, nuclear membrane. *D*, nucleus, filled with karyoplasm. *E*, nuclear reticulum, composed of linin and chromatin. *F*, plastid. *G*, metaplastic inclusion. *H*, chondriosomes. *I*, vacuole. *J*, membrane of vacuole. *K*, cytoplasm. *L*, ectoplast or limiting membrane of the cell. (From Sharp "Introduction to Cytology," McGraw-Hill Book Co.)

The Characteristics of Cell Structures.—Typical cells possess a cell wall, cytoplasm and nucleus. Figure 47 is a diagrammatic representation of these and other typical cell structures.

The *cell wall*, as stated above, is not characteristic of all cells; but when it is present, it serves to retain the definite form of the cell and to

protect it. Some cells that have definite walls are shown in Fig. 45. Cells that do not possess a cell wall, for example, amoebas, other rhizopods and leucocytes, are provided with a plasma membrane, whose formation and composition were discussed in Chap. III. This plasma membrane appears to be characteristic of all protoplasm, for it forms the surface of the true living matter even in cases where the cell wall is of rigid form, as for example, in plant cells. The significance of the protective and selective powers of the plasma membrane has already been discussed (pp. 74-88).

The *cytoplasm* of cells cannot be briefly characterized because it is so different in different types of cells. The word cytoplasm refers to the same concept that was formerly represented by the word protoplasm. It was then supposed that the nucleus of a cell was not composed of living matter. Since this assumption now seems unwarranted, all the typical contents of cells are included in the term protoplasm, and the nuclear material is separately designated as *karyoplasm*, other protoplasmic material as cytoplasm. Cytoplasm means, then, all the living substance inside the cell boundary, with the exception of the nucleus. As the latter is commonly distinguished by its ability to take up certain dyes, the practical definition of cytoplasm is: The portion of cell contents that does not stain with the so-called nuclear stains. Typical cytoplasm is sometimes said to be a transparent, viscid, microscopically homogeneous, colorless solution and is exemplified by the clear portions of an amoeba near its margins and in smaller pseudopodia; but it is doubtful if such transparent material is really typical of cytoplasm. Most cells, indeed, show granules or other structures within their cytoplasm, that give it a heterogeneous appearance even under comparatively low-magnification and even the clearest specimens of cytoplasm are micro-heterogeneous under the ultramicroscope. All cytoplasm doubtless contains some comparatively transparent material constituting the main dispersing medium or "groundwork" in which the more heterogeneous and structurally defined material is suspended; but the latter has come to be regarded in recent years as constituting part of the living cytoplasm just as truly as do the more fluid and more homogeneous portions.

The *nucleus* is generally a distinct structure enclosed in its own membrane. Basic stains are used to distinguish nuclear material for microscopic examination. Hematoxylin is frequently employed. These stains are used on cells that have been previously treated with a killing and fixing solution because satisfactory dyes for "vital staining" of nuclei are not available. Staining processes have come to be depended on to distinguish nuclei, for the reason that these structures and, indeed, all colorless structures in the cell are not clearly visible, even with high magnification, in their natural state. This is due to the fact that all the gel-like, semi-transparent materials, of which these structures are com-

posed, have nearly the same index of refraction and therefore do not show distinct outlines in the field of the microscope. This has necessitated the development of elaborate methods of cell staining. By these methods and also by such observations as can be made on living cells, it has been shown that the nucleus is, in some cases, a single distinct body, located near the center of the cell; in other cases, each cell has numerous small nuclei, irregularly located; in rare instances, the nuclear material is not definitely located but is scattered in a diffuse condition throughout the cell. Karyoplasm, like cytoplasm, appears to consist of a comparatively transparent and more fluid material which surrounds more definite structural bodies such as chromatin threads, or *chromosomes*, and the *nucleolus*. The latter is not seen in the nuclei of all cells.

Numerous special structures are found in the cytoplasm of certain cells. The *chromatophores*, containing the chloroplasts (p. 4) are special structures of green plant cells. The *fibrilae* of muscle cytoplasm are specialized structures of animal cells. Minute granules are generally present in cytoplasm. In many animal cells some of the granules consist of glycogen as demonstrated by their ability to stain red with iodine. Some of the granular material consists of phospholipins, especially in cells of nervous tissue. This fact has also been established by microstaining tests. Proteins are supposed to exist in granules; but owing to the general distribution of proteins throughout cytoplasm, staining reactions do not especially distinguish the protein material of the visible structures. Granules are, in many cases, at least, aggregates of storage material in the cell. For example, the granules that contain glycogen in liver cells are carbohydrate storage material in compact form. The so-called *zymogen granules*, that are found in various gland cells after a period of inactivity, doubtless represent stored up materials. This seems to be the case because they largely disappear during the time that the gland is actively secreting. Some of the special structures of the cell, such as chloroplasts and fibrilae are permanent and should be regarded as special organs of the cell with definite functions to perform; while other cytoplasmic structures, such as granules are usually temporary and should be regarded as deposits of certain cell constituents in comparatively concentrated form.

Analogous to granules are the starch grains of plant cells and the droplets of emulsified neutral fat or large globules of fat, as found in many types of cells under conditions of abundant nutrition. Starch and neutral fats, as explained above, are storage materials of the cell.

Other cytoplasmic structures, in addition to masses of storage material, appear temporarily in cells. One of these is the *aster*, (Fig. 47). This structure consists of a nodule called the *centrosome* and strands which radiate from the centrosome. Asters function in cells during their multiplication by the process known as mitotic division or *mitosis*. In this process, two centrosomes, present in cells that are about to divide,

develop radiating strands and thus assume an aster-like appearance. The two asters so move, during their development, that they take up positions on opposite sides of the nucleus. Certain of their strands then become arranged in a spindle which includes the nucleus. The strands appear to become attached to the chromosomes of the nucleus whose nuclear membrane has meanwhile disappeared. By contraction, the

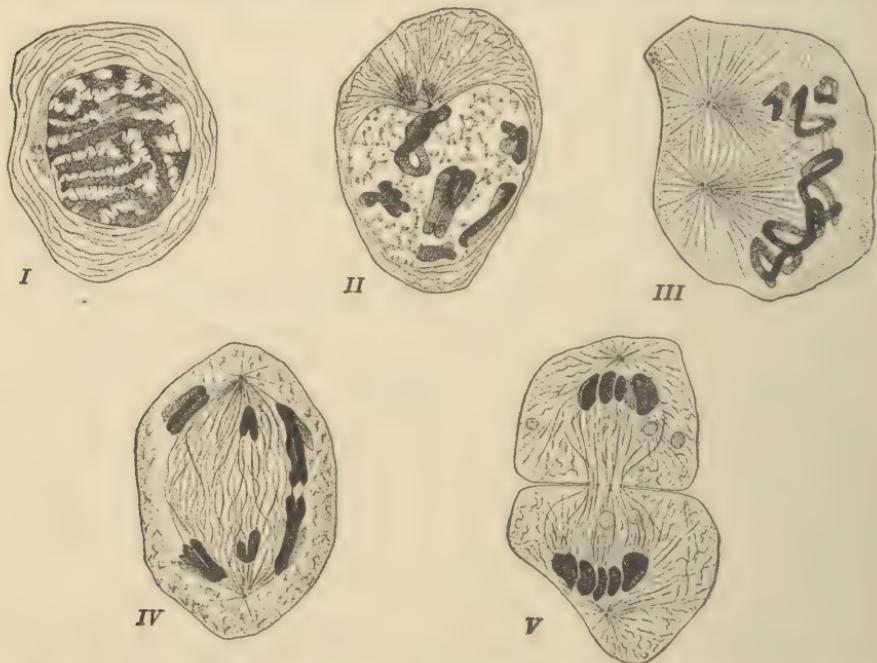


FIG. 48.—Mitosis in the spermatocyte of *Salamandra*. I, a stage preceding the more active part of mitosis. The centrosomes, shown at the left of the large nucleus are inconspicuous and without visible asters. II, a later stage, chromosomes separated and asters developed. III, a further stage, the chromosomes attached to the threads of the asters which have formed a spindle between them. The nuclear membrane has disappeared. IV, a still later stage, chromosomes drawing toward the centers of the asters. V, mitosis nearly complete, with constriction to form two cells. (From Sharp "Introduction to Cytology," after Meves.)

strands split the chromosomes and guide half of each chromosome into one of the two cells that now form by means of a dividing constriction of the original cell. Figure 48 shows mitosis as it occurs in certain types of isolated cells.

Another structure that occurs temporarily in many cells is the *vacuole*. As formed in the amoeba, for example, it has the appearance, represented in Fig. 47. Vacuoles are produced around ingested foreign materials or as the result of segregation of cellular waste products. Vacuoles may contain gas or liquid but in any case are surrounded by a protoplasmic membrane, through which waste products or digestive enzymes may be secreted by the cytoplasm into the vacuole while food

substances may be absorbed into the cytoplasm. The vacuole is, then, a temporary digestive organ of a cell. The vacuole functions as an excretory organ in motile unicellular organisms, since its contents can be extruded from the cell by means of a suitable streaming of the cytoplasm.

Cytology and Physical Chemistry in Relation to Physiology.—The visible structures of cells, membranes, clear fluid portions, chromosomes, chloroplasts, fibrils, granules, fat emulsions, etc., have been studied extensively and described with more and more accuracy during the past century. The physiological significance of each of these structures has also been investigated. These studies constitute that branch of biology, known as *cytology*. Although this science has of necessity been largely descriptive and, partly on that account, more closely allied to anatomy and histology than to physiology, cytology has now reached a stage where it joins forces with biochemistry and biophysics. For these studies have also developed through stages that consist of little more than description of static conditions and, like cytology, have acquired the technique and formulated the quantitative laws which make possible an investigation of dynamic conditions in protoplasm, that is, of the nature of vital processes. The time appears to be ripe for the discovery of facts that have a deep significance for the explanation of the nature of life. Many disclosures have already served to blaze promising paths into the maze of cellular complexities. Such pioneer work has been especially conducive to further investigations in those instances where it has employed the methods of physical chemistry. This has been the case, because vital processes are particularly characterized by the types of reversible changes that are fundamentally physical rearrangements. These, in effect, amount to chemical changes; but being of an easily reversible and distinctly unstable nature, they are not always detectable either by strictly chemical or strictly physical methods.

Several of these physico-chemical processes and the forces causing them have been described in preceding chapters: (1) Surface tension as a force that partly determines the distribution of substances in protoplasm (pp. 77 to 81), (2) the antagonistic action of salts as a force that can determine the nature of an emulsion (pp. 81 to 87), (3) the especial ability of the carbon atom to form asymmetric combinations which confer highly specific properties to the resulting compounds (pp. 17 to 18), (4) the physico-chemical properties of water that render it especially suitable as a solvent for the diverse and reactive materials of protoplasm (pp. 133-137), (5) osmotic pressure as a force controlling the movements of water across semipermeable membranes, (6) electrolytic dissociation as the process which enables the resulting electrically charged ions to be especially reactive chemically (pp. 132, 133), (7) the peculiar powers of H- and OH' ions in influencing hydrolyses and, indeed, all biological reactions (pp. 172, 183), (8) the equilibrium of chemical reactions (pp. 184, 190), (9) the catalytic action of enzymes and the conditions which determine the equilibrium of enzyme reactions (pp. 228, 229). Other matters of similar physico-chemical character have also been investigated. The following sections deal with certain aspects of physical chemistry that have not been considered above.

The Physico-chemical Condition of Protoplasmic Constituents.—Protoplasm is generally described as a foam (emulsion) structure. The word foam is misleading in that protoplasm does not usually contain gas in the form of bubbles which compose the dispersed phase of a foam. Protoplasm is characterized more correctly as an emulsion structure. This characterization is based upon observations of living cells and is in accord with the idea of the colloidal character of protoplasm as outlined above. Detailed studies of living cells show, however, that the discernible globules, that give protoplasm its foamy appearance are not necessarily uniform in size, nor are they homogeneous within each globule. In other words protoplasm is not of the character of an ordinary emulsion. Some of the material of the cell may, to be sure, constitute a comparatively simple emulsion, as happens when fat deposits occur in certain types of cytoplasm. But for the most part, protoplasmic materials are in a much more finely divided state than are the substances of an ordinary emulsion.

Emulsifiers of Protoplasm.—Even substances of a fatty nature that are particularly apt to form emulsions *in vitro*, do not always occur as distinguishable bodies in cells. Staining methods for the detection of fats may fail to reveal any fat in certain cells and yet a mass of them may yield a large quantity, as much as 5 per cent of the weight of the cells, to ether extraction. This means that fatty materials of such cells are in a state of ultramicroscopic subdivision. The emulsifying agencies of protoplasm must be highly efficient. They consist, doubtless, of proteins and soaps in most cells. As stated above, bile salts are the most effective emulsifiers found among biological materials, but they are produced only in the liver, and do not find entrance into most cells. Soaps, however, might well be produced in minute amounts in protoplasm, because living matter is generally alkaline and fats or lipoids that yield fatty acids are always present. These conditions favor the formation of at least a trace of soap and only a minute amount of it is required for emulsification of fats. Protein solutions and fats are also capable of forming fine emulsions. Combinations of fats, soaps and proteins produce stable and complex colloidal forms. Lecithins form fine emulsions without the aid of any special emulsifier. Indeed, lecithins may become so finely dispersed in water that their particles, like those of dissolved proteins, are submicrons or amicrons.

Given, then, fats and their emulsifiers (soaps and proteins) together with lecithin and proteins in colloidal solution and given also inorganic salts whose effects on emulsions and other colloidal states vary with their relative concentrations, it is not difficult to picture some of the numerous possibilities of microheterogeneous structures that doubtless occur in protoplasm.

Films.—The manner in which such colloidal combination can produce a definite visible structure is shown during the heating of milk. Milk contains proteins and lipoids in colloidal solution, fats in colloidal suspension (emulsion), and other substances in true solution, among them inorganic salts, especially calcium phosphate. Milk, on heating, produces a surface film of membrane-like character and if this film is lifted off another forms in its place. Such films, when chemically analyzed, are found to be composed chiefly of milk fats, casinogen and calcium. The formation of this film appears to be analogous to production of membranes in protoplasm and, indeed, the physico-chemical forces that produce it are doubtless the same ones that operate in cells. Moreover, protoplasmic surfaces possess a distinct, though often invisible membrane.

Kite has shown that the surface layer of an amoeba, removed by micrdissection, can be cut into small pieces that have a gel consistency. The greater complexity of composition of protoplasm as compared with milk renders the analogy incomplete; but at least it shows that a slight change in temperature (the film does not form at 40°C. but does at 45°C.) can markedly alter the state of aggregation of fats and proteins in the surface layer of a complex emulsion. As protoplasm is also greatly altered by a similar change in temperature, the analogy is striking. This film production is also markedly influenced by hydrogen ion concentration. If milk be changed from its normal pH of approximately 6.7 to a pH of approximately 4.7, film production does not occur on heating, but clotting of the milk (precipitation of casinogen), occurs instead. In this respect, then, the complex emulsion in milk also becomes analogous to that of living structures, for they, too, are very sensitive to changes in C_H^+ .

Another useful illustration of film production in colloidal solutions is the formation of protein precipitation membranes. Harvey has shown one method for the production of such membranes. His method is to shake up chloroform in a solution of egg albumin. Microscopic drops of chloroform are produced and each one is surrounded by a precipitated protein film which is comparatively stable. The film must be of ultra-microscopic thinness. Even the highest magnification fails to show it; but its presence is proved by the behavior of the globules. They can be washed with water until no further removal of albumin can be effected and still each globule remains distinct with chloroform held within it. In short, a distinct surface film apparently protects each droplet. This film can be made visible if alcohol is added to the water surrounding the droplets, for this treatment causes them to burst open leaving microscopic films of alcohol-coagulated protein. The films must be present upon the chloroform drops, even before they are made visible by alcohol, because otherwise the drops would coalesce as they do after chloroform is shaken up with pure water. Another proof of the presence of the film

is seen in its semipermeable behavior. If the chloroform droplets are immersed in carbon disulphide or xylol the droplets are seen to shrink and take on an irregular wrinkled shape like a crenated corpuscle or a plasmolyzed cell. The film thus behaves like the plasma membrane of a cell immersed in a hypertonic solution. The protein film of the droplets is more permeable to chloroform than to carbon disulphide or xylol, so that chloroform can be drawn out of the droplet by osmotic pressure.

Not only albumin, but several other proteins, for example, gelatin, have been used in the preparation of these enfilmed chloroform droplets; so that the property of film formation doubtless belongs to all protein solutions. Moreover such films are producible upon diverse kinds of surfaces. The comparative stability of droplets of emulsified fat in protein solutions is evidence that proteins form films upon fat in the same manner that soaps do. Protein solutions, in contact with alkaloidal precipitants, such as tannic acid, produce precipitation films of great stability and tenacity. Thus it is entirely probable that protoplasmic films of protein, or at least films containing protein, occur naturally in animal and plant cells.

The visible films, such as enclose the nucleus of most cells, and the ultramicroscopic films, whose existence upon the outer surface of protoplasm is indirectly demonstrated by the semipermeability of cells, have been especially studied. But there is physico-chemical evidence to show that similar films occur wherever two dissimilar substances come in contact; so that the chromosomes of the nucleus, the fibrilae, granules and other structures of the cytoplasm, the droplets of emulsified fat and even the globules that have been described as imparting a foamy structure to cytoplasm are probably all surrounded by films, composed of substances that belong to the film-forming groups of compounds: Proteins, lipoids, soaps and inorganic salts.

The differing permeability of these films is supposed to impart to protoplasm its ability to carry on different chemical reactions in specific parts of the cell. A cell has been compared aptly to a well organized factory of which the separate working units represent the different compartments of protoplasm. Just as efficient production requires that specialized processes should all proceed separately yet coordinatedly in the different shops of a factory, so the vital processes require that specialized reactions proceed side by side in a coordinated manner in protoplasm. This analogy helps one to understand why any mechanical disruption of the structural organization of protoplasm results in abnormal metabolism or in death.

Viscosity.—Not only film-forming power but other properties of protein solutions contribute to the organization of living matter. Typical protein solutions behave as though the dissolved molecules possessed a certain cohesion for each other or for the solvent. This cohesion is

inferred from the marked viscosity of protein solutions. Viscosity can be measured in terms of the time required for a given solution to pass through a capillary tube of given bore under given conditions of temperature and pressure. Such measurements show that protein solutions of comparatively low concentration possess viscosities of the same order as that found for high concentrations of many other substances. Viscosity is really the resistance of a solution to change of shape and is said to be due to "frictional resistance" to the movement of molecules past each other. Such an interpretation of viscosity is at least applicable to non-colloidal solutions or to suspensoid colloids. But the viscosity of a typical protein solution or a protein gel must be of a peculiar character because it presents little more impediment to the diffusion of small molecules and ions than does pure water itself. This fact is shown by the rapid rate at which colored substances can be seen to distribute themselves throughout a viscous protein solution or a gel. Another demonstration of this fact is obtained by the measurement of electrical conductivity of salt solutions, for such measurements show that the conductivity of a water solution of a salt is scarcely diminished by the addition to it of a highly viscous protein. If the same viscosity were produced in the solution by the addition of glycerol or sugar, electrical conductivity would be greatly diminished. Since electrical conductivity in a solution, containing a given concentration of ions, can vary, at constant temperature, only as the rate of migration of ions varies these observations show that protein viscosities do not impede ion movements. These facts suggest that protein solutions have their protein molecules so arranged that cohesional forces connect them somewhat as the strands of a three-dimensional network would connect the knots of the net. Such a net would not impede the passage of particles smaller than its meshes (diffusion of small molecules and ions), but would resist a deformation such as would be required to force it through a narrow tube, that is, would show high viscosity. This is the view presented by Robertson. It appears to apply to protein gels, at least. Loeb has shown that the viscosity of protein solutions, as contrasted with that of gels, may be accounted for by means of the relative volume which protein particles occupy in their solutions. According to this idea, each protein particle has a certain structure which enables it to hold many molecules of water within it. But in either case, the viscosity of proteins is attributable to the structural organization of their molecules. Viscosity of protoplasm is due chiefly to proteins. The inference is that there is a structural organization of protein molecules throughout even the comparatively homogeneous and more fluid portions of protoplasm.

The physiological significance of this property of proteins is that it doubtless explains how protoplasm can maintain a comparatively stiff, semi-solid, gel consistency in portions of its structure without checking

the diffusion of molecules and ions or the reactions that depend upon such diffusion.

Summary.—The outstanding fact concerning the physico-chemical condition of protoplasmic constituents is that they are in an emulsified state. This is the central idea of the foam (emulsion) theory of protoplasmic structure. But in addition to microscopically visible emulsification, protoplasm is further subdivided within its visible phases in that some of its material is ultramicroscopically dispersed in very fine emulsification. This emulsified condition favors the production of membranes which surround or divide the protoplasm. Many of these membranes are mere films of ultramicroscopic thinness. There is still another type of minute structure within the phases of protoplasmic emulsions, due to the peculiar type of cohesion between protein molecules. It is this cohesion which gives protoplasm its viscosity.

Factors Affecting the Physico-chemical condition of Protoplasmic Constituents.—The consistency of protoplasm is subject to distinct changes. Its viscosity in a given portion of the cell may increase or decrease and in most cases these changes are reversible. The amount of the change may be so great as to cause a distinctly fluid consistency to change into the state of a stiff gel. Numerous examples of such changes have been observed. Bayliss has shown that in an amoeba, granules, lying just inside the clear outer layer of cytoplasm, are normally in rapid Brownian movement which proves that this portion of the cytoplasm is in a liquid state; but when the amoeba is subjected to an electrical stimulus, the Brownian movement suddenly ceases as though the granules were held in a stiff gel. The movement is resumed when the stimulus stops, indicating that the cytoplasm is again liquid. The gelling process thus appears to be reversible. Another example of change in consistency is seen in cells during the processes of division. Chambers and others have shown that eggs of sea-urchins and other echinoderms change, during each cell-division, from a comparatively fluid condition to a more viscid (gel-like) state and that this change is reversible. L. Loeb has shown that the blood cells of the horse-shoe crab (*Limulus*) produce pseudopodia of distinctly different viscosity. Their forms vary from sharp, pointed or threadlike processes to blunt, rounded protuberances. The former are highly viscid, the latter of a fluid, soft consistency. These variations can be produced at will by the experimenter through changes in the osmotic pressure of the surrounding fluid, or changes in temperature, or the concentration of potassium ions. As similar variations in the character of the pseudopodia or of the outer layers of numerous types of cells have been recorded, changes in the consistency of cytoplasm probably occur in many different kinds of cells.

An explanation of the cause of these changes in cytoplasmic consistency cannot be given completely; but numerous observations, that have

been made in researches on the behavior of colloids, seem to have a distinct bearing upon this matter. Of such observations, those relating to the changes in the behavior of proteins, in response to changes in hydrogen ion concentration, are especially significant. J. Loeb has shown, for example, that gelatin has entirely different viscosity, solubility, powers of swelling in water and entirely different combining powers at different hydrogen ion concentrations. These differences in the behavior of gelatin are due primarily to the differing powers of electrolytic dissociation of the protein at different hydrogen ion concentrations.

In sufficiently acid solutions gelatin dissociates as a base and is able to make combinations with acids to form such compounds as gelatin chloride, gelatin sulphate, etc. In slightly acid, neutral or alkaline solutions, however, gelatin can dissociate only as an acid; so that it forms gelatinates, such as sodium gelatinate, calcium gelatinate, etc. At a certain C_H ($pH = 4.7$) gelatin does not dissociate detectably, either as an acid or a base. This point is known as the isoelectric point and is so named under the assumption that, if gelatin dissociates at all at its isoelectric point, it dissociates to an equal extent both as an acid and a base. It probably does not dissociate at all, because solutions of gelatin, having $pH = 4.7$, may be subjected to an electric current without causing any migration of gelatin toward either anode or cathode, whereas in solutions of gelatin, possessing pH values lower than 4.7, it accumulates at the cathode and in solutions of gelatin, possessing pH values higher than 4.7, it accumulates at the anode. Similar isoelectric points have been determined by other investigators for other proteins. Indeed, every amphoteric substance should, theoretically, show an isoelectric point. But the isoelectric point of proteins has a peculiar chemical significance because the properties of proteins, vary so widely with changes in C_H near the isoelectric point.

The variations in the combining powers of proteins, on either side of the isoelectric point, are well illustrated in Loeb's experiment on gelatin as shown in Fig. 49. In this experiment silver nitrate was permitted to react with a given quantity of gelatin suspended in each of a series of solutions each of which had been brought to a definite pH by the addition of the proper amount of acid or alkali. The excess of silver nitrate was removed from the gelatin by washing upon a filter and the gelatin was subsequently dissolved. The resulting solutions are shown in the illustration which clearly indicates that those solutions on the less acid side of the isoelectric point have a color differing from that of the solutions on the more acid side of the isoelectric point. The former have turned dark because they contain silver, which has been liberated by the action of light; while the latter are nearly colorless and contain no silver. The latter can combine, however, with bromine as was shown by another experiment. Moreover, the proportions of silver combined with gelatin

shows a regular increase with increase in the pH of the solutions, while the proportion of bromine in combination with gelatin on the other side of the isoelectric point regularly increases with decrease of the pH of the solution. At the isoelectric point, neither silver nor bromine combines with gelatin. Not only silver ions, but ions such as sodium, potassium and calcium, indeed, all cations, are similarly limited by hydrogen ion concentration in their reactions with proteins, while all anions are limited in the same way that bromine is in making combinations with gelatin.

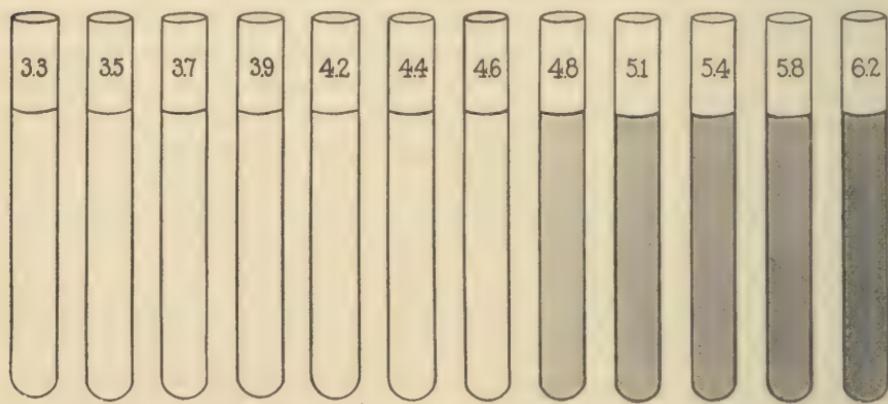


FIG. 49.—Proof that cations combine with proteins only on the alkaline side of the isoelectric point. Powdered gelatin brought to different pH was treated in a dark room with M/64 AgNO₃ and then washed with cold water to remove the silver not in combination with gelatin. The gelatin was liquefied, brought to a 1 per cent solution, and the pH was determined. The solutions were then poured into test-tubes and exposed to light. In about half an hour the gelatin of pH > 4.7 was dark while the gelatin of pH 4.7 or less remained permanently clear though exposed to light for over a year. The pH of each gelatin solution is marked at the head of each test-tube. (From Loeb's "Proteins and the Theory of Colloidal Behavior," McGraw-Hill Book Co.)

The experiment with silver bromide is chosen for illustration merely because the results are more easily and strikingly demonstrated.

Parallel to the influence of hydrogen ion concentrations upon protein combining powers is its influence upon protein viscosity and solubility, and upon the tendency to attract water, as shown by the swelling of gelatin. Curves showing the effects of hydrogen ion concentration upon viscosity and swelling are given in Fig. 50. The sharp minimum of these properties of the protein at the isoelectric point is striking.

Changes in viscosity of cytoplasm might readily be explained on the basis of the above facts, that is, on the basis of changes in the hydrogen ion concentrations of different portions of the cell and consequent alteration in protein viscosity. The difficulty in accepting such an explanation is that such changes in hydrogen ion concentrations have not been demonstrated in cytoplasm under normal conditions, nor have the isoelectric points of the proteins in cytoplasm been determined. But

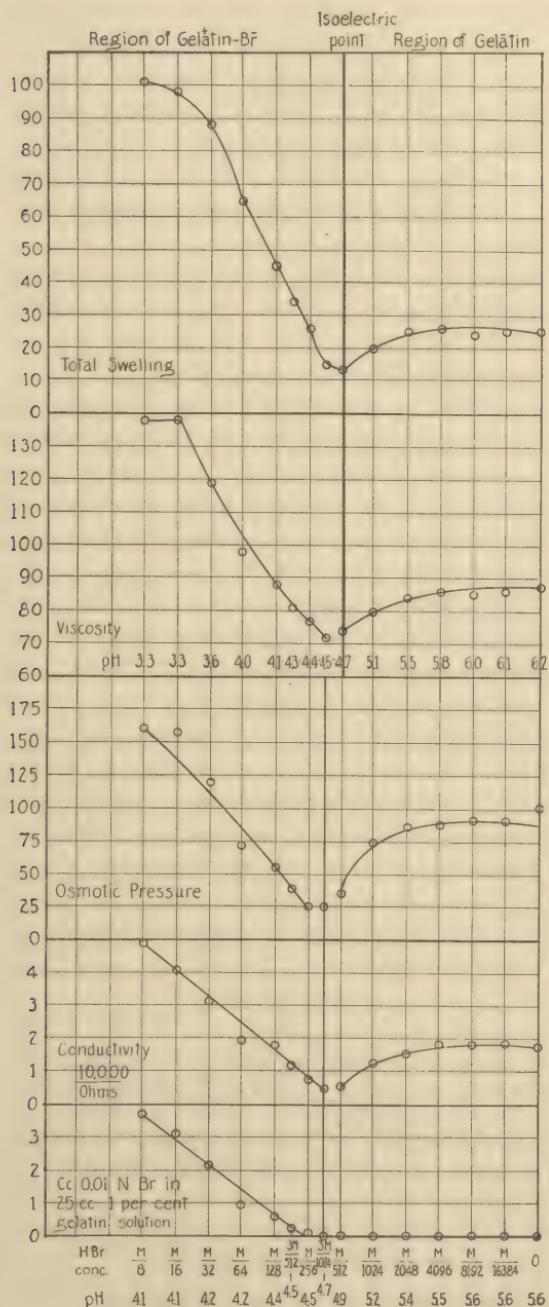


FIG. 50.—Showing that the physical properties of gelatin are a minimum at the isoelectric point. (From Loeb's "Proteins and the Theory of Colloidal Behavior," McGraw-Hill Book Co.)

exposure to acid media will markedly change the viscosity of plant and animal cells, so that their protoplasmic fluidity may be increased or decreased at will and such changes are reversible. Moreover, the acid most commonly produced in living cells, namely carbon dioxide can produce this effect. The viscosity changes, occurring in normal cytoplasm, however, are highly localized in certain definite parts of the cell so that if these changes are caused by changes in C_{H^+} , the latter must be definitely localized.

Changes in the distribution of water in different portions of the cell might also be explained on the basis of localized changes in the hydrogen ion concentration of cytoplasm, since the attractive power of proteins for water varies so markedly with changes in C_{H^+} .

Changes in the state of aggregation of proteins in cytoplasm might be explained in the same manner, because the electrolytic dissociation and therefore the dispersion of proteins in solution are markedly altered by slight changes in C_{H^+} . At the isoelectric point many proteins form insoluble flocculent masses or even hard compact granules; but at hydrogen ion concentrations on either side of the isoelectric point such proteins dissolve, with electrolytic dissociation, so as to produce the conditions requisite for chemical reactions of proteins.

Since the combining powers, the viscosity, the attraction for water, the solubility, the electrolytic dissociation and the state of aggregation of proteins are all markedly influenced by hydrogen ion concentration and since proteins are among the most important and abundant constituents of all living matter, the regulation of the C_{H^+} of the cell and its environment by buffer substances, as described, has a peculiar significance. These facts of protein chemistry also help to explain why any change in C_{H^+} of living matter, except within certain narrow ranges, causes marked abnormalities of physiological behavior or even death.

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CHAPTER XI

EXCITATION AND INHIBITION

An attempt to decide what constitutes the difference between living matter and dead matter cannot avoid the observation that one especial characteristic of living matter is its power to react to changes in its environment. Every living thing responds by some reaction to various physical or chemical changes in its environment. If an animal is alive it responds to certain changes in pressure, heat, light etc., by movements; if a seed is alive it responds to increase in moisture and temperature by germination; if anything is alive it will show some kind of physiological activity in response to some appropriate environmental change. This responsiveness is sometimes called the irritability of living matter, though the term excitability is preferable.

Dual Control.—The response may take the form of an increase of some kind of activity, such as motion, growth, secretion, etc., or it may take the form of a decrease of these activities. If the response is an increased activity, the protoplasm is said to be excited; if the response is a decreased activity, the protoplasm is said to be inhibited. This dual responsiveness, which is found in numerous types of cells, would seem, at first thought, to be unnecessary; because decrease of activity would naturally follow any decrease of excitation. But just as the brakes of an automobile greatly increase its controlability, so inhibitions of living mechanisms make them respond sensitively to environmental changes. An environmental change causing excitation or inhibition is called a stimulus.

The Response.—The nature of the response is not always the same, even though the stimulus is identical; for the response depends upon the structure of the stimulated cells. Muscle cells respond by contraction; gland cells by secretion; nerve cells and their branches, nerve fibers, by conduction of nerve impulses; certain cells of the sensitive plant by changes in shape so as to cause drooping of the leaves; cells of all plants respond by changes in growth or other metabolic changes and by changes in the movements of their protoplasm. The responses to stimuli are often very complex in the higher animals, especially human beings, because even a localized stimulus can cause different kinds of cells to be excited or inhibited by means of the correlating action of the nervous system. But in any case the nature of the response to stimuli is of the same general character in any given kind of cell, no matter what the

nature of the stimulus may be, provided it acts in a normal or physiological manner, and is applied to the same part of the cell.

The Independent Excitability of Muscle.—The universal excitability of living matter is not always apparent. For example, skeletal muscle might be regarded as responding only through the mediation of the nervous system since a muscle appears to be completely paralyzed when its functional connection with the nervous system is severed by nerve section or degeneration. A skeletal muscle, thus isolated, does indeed become useless and even degenerated from disuse. But this result is due to the fact that stimuli can no longer reach it effectively and not to a loss of excitability in the muscle cell; for if an electrical stimulus be applied to such muscle directly, or to the skin in such a way that the current passes through the muscle, a normal contractile response is obtained.

The famous experiment of Claude Bernard may also be used to demonstrate the independent excitability of muscle. In this experiment, usually performed on a frog, the animal is injected with a small amount of the arrow poison concocted from plant juices by Indians of South America and called curare. This poison causes complete paralysis of the skeletal muscles because it acts upon some substance, lying between the ends of nerve fibers and muscle protoplasm, so as to temporarily but completely inhibit the normal power of this substance to conduct nerve impulses. As a result of this action muscles of the poisoned animal, said to be curarized, cannot be made to contract by stimulation of the nerves. If, however, the muscles are directly stimulated, as, for example, by applying electrodes from an induction coil, the muscles contract with normal vigor. This proves the independent excitability of muscle and also shows that curare does not poison the contractile substance of muscle. The fact that curare does poison the substance between nerve and muscle is proved by experiments in which the circulation to a given muscle or group of muscles is tied off before injection of curare. In this case the poison has access to all the nerves leading to the ligated region but not to the endings of these nerves. It is then found that stimulation of these poisoned nerves arouses impulses in them and produces normal contractions of the muscles in the ligated, non-poisoned region, although all the other muscles of the animal are completely paralyzed. Inasmuch as an inhibiting action of curare upon the true muscle substance and upon the nerve fibers can thus be excluded, the inference is that its action is exerted upon something between nerve and muscle.

Excitability of All Living Matter.—Because of this and many other similar observations, excitability has come to be regarded as an unfailing attribute of all living matter. The term excitability or irritability is sometimes used in a restricted sense to refer to the property of muscles, nerves and a few other structures that respond to weak and diverse kinds of stimuli by showing especially rapid effects. Such structures are some-

times called the excitable (irritable) tissues. But modern opinion regards this distinction as only a matter of degree of excitability and as probably due to the manner in which the especially excitable portion of the protoplasm is placed in the cells. Every living cell is doubtless capable of responding to the right kind of stimuli properly applied. Yet one may well say that this property is not confined to living matter alone. True, mechanical systems are responsive to change in pressure, and other mechanical conditions; every non-living substance gives some kind of a response to changes in temperature; numerous chemical reactions are stimulated by light; motors and chemical reactions are set going by elec-

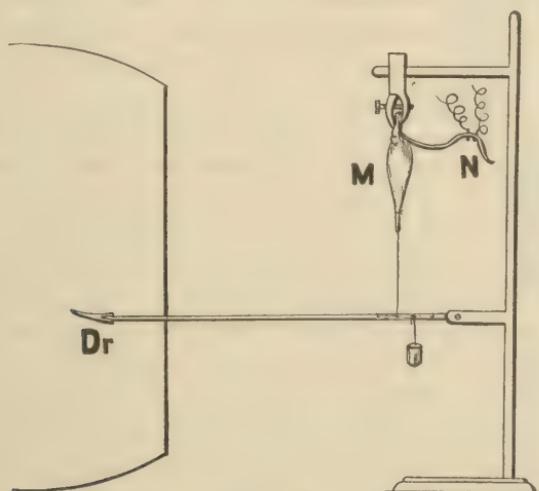


FIG. 51.—Frog muscle-nerve preparation. *M*, gastrocnemius muscle with tendon attached by a thread to a weighted muscle lever. The femur to which the muscle is still attached is held by a clamp. *N*, sciatic nerve in contact with the electrode wires of a stimulating apparatus. *Dr*, drum upon which the movements of the muscle lever point are recorded.

tricity. But no artificial physico-chemical system (mechanism or combination) shows responsiveness to so great a diversity of physical and chemical forces as does living matter and the latter is more particularly characterized in that it responds to exceedingly minute changes in pressures, temperatures, light intensities, concentrations of effective substances and other environmental conditions. The responses of living organisms to certain physiological reagents are far more sensitive than are the most delicate chemical tests. Examples of such sensitiveness are the stimulating action of Fe, Zn and Mn upon plant growth (p. 118) and the stimulation of the sense of smell of a bloodhound when it trails a given individual by the scent of footprints.

The Frog Muscle-nerve Preparation.—To a large extent, the facts of excitation have been established by experiments on the frog muscle-nerve preparation. As its behavior aptly illustrates many of the phenom-

ena, about to be discussed, a description of this preparation is necessary at this point. The gastrocnemius muscle and sciatic nerve constitute the preparation and have been widely used because this muscle is easily removed intact with the tendon of Achilles attached to its lower end and a portion of the femur attached to its upper end, while the nerve can also be dissected out so as to yield a conveniently long stretch, extending from the pelvic plexus to the point where the nerve enters the gastrocnemius muscle. The completely excised muscle may be suspended without injury by a clamp attached to the fragment of femur and the tendon may then be attached to a suitably weighted lever without any injury to the true muscle substance (Fig. 51). As the movements of the lever magnify any contraction of the muscle, all responses to stimuli, applied to either nerve or muscle, are easily observed.

Several advantages in the use of frog muscles have made this preparation especially useful. Frogs are commonly available and can be easily kept in the laboratory for months without feeding. The dissections required for excising the preparation can be conveniently made on the living animal without the necessity of anaesthesia, because all sensibility is easily destroyed by the procedure known as *pithing*. This consists in destruction of the brain and spinal cord by thrusting a blunt needle into the cranium and into the spinal canal. For each of these brief operations, the needle is inserted through the same opening, a small cut made through the skin and other tissues which lie dorsally over the opening, between the cranium and spinal column, at the base of the skull. The accumulated store of nutrients in frog muscle permits use of the isolated preparation without the necessity of providing it with a nutrient solution. As the frog is poikilothermic (cold-blooded), its muscle is adapted to show irritability over a comparatively long range of temperature; so that a thermostat for maintenance of constant temperature is not required in experiments on frog muscle. Other excised muscles of the frog, especially the sartorius, also muscles of other cold-blooded animals, for example, the foot muscle of the slug, and even excised muscles of warm-blooded animals have been employed in physiological experimentation; but no excised preparations have as many advantages as the gastrocnemius-sciatic of the frog.

Certain precautions are required for satisfactory use of the muscle-nerve preparation. Drying of the tissues must be prevented. This is insured by keeping the preparation moistened with isotonic salt solution. Water would, of course, cause disastrous changes in osmotic pressure. Ringer solution has obvious advantages for the purpose, because it preserves the balance of the concentration of physiologically important inorganic ions (Chap. VI). If immediately after the preparation is dissected out, it is thoroughly moistened with Ringer solution, it may be conveniently kept in an excitable condition during a prolonged experiment by enclosure in a moist chamber which prevents evaporation. Another necessary precaution is to the use of only those exciting stimuli which do not cause destructive effects upon the tissues. This requirement is met by the use of electrical stimuli, applied to the tissues through non-polarizable electrodes, described below.

The Graphic Method.—Exactness of physiological observations has been largely dependent upon the use of the graphic method. In this procedure, movements of levers, whether attached to muscles or to other moving mechanisms, are recorded. The point of the lever is made

to bear with the least possible friction upon a recording surface. As the lever moves up or down, its point traces a curve upon the simultaneously moving surface. For this reason the recording apparatus is called a kymograph (curve writer). The form devised by Ludwig and modifications of it (Fig. 52) are widely employed in physiology. They have a metal cylinder, called the drum, which is covered before use with glazed paper, upon which is deposited a thin layer of soot by rotating the drum over a smoky flame. Smoked, glazed paper offers a minimum of friction to the movements of the writing point. Any smoothly running motor can be used to impart a uniform motion to the drum. Clock work is commonly employed. Abscissae of the kymograph curve are time intervals and it is usually necessary to make a simultaneous time record during the tracing of the curve. For this purpose a writing-point bears upon the drum directly below the point of the muscle lever and is given suitable vertical motion at definite time intervals by clock work or by an electromagnet in circuit with a vibrating tuning fork or pendulum. The smoked drum kymograph record is made permanent by passing the paper, bearing the completed curve, through a bath of white shellac.

More recent developments of graphic recording have perfected the photographic method. In this method, the levers or other moving parts of apparatus are placed in the path of a bright light in such manner that their shadows are focussed by lenses upon the sensitized surface of a moving photographic plate or camera film that is made to move past the camera slit at a uniform rate by means of a suitable motor. Certain types of physiological investigation, for example, those depending upon records of the movements of a string galvanometer, were made possible by development of the photographic method of recording.

The Nature of Stimuli.—Inasmuch as living matter is very responsive in its phenomena of excitability, almost any environmental change may constitute a stimulus under some circumstances, at least for certain organisms. Examples of stimuli are: (1) Electrical changes, that is, any change in the state of rest or motion of electrons; (2) pressure changes, that is, mechanical stimuli; (3) electromagnetic changes as seen in the action of light, of ultraviolet rays, of X-rays, of radio activity, etc.; (4) chemical changes, such as changes in the abundance of food or water or substances of a foreign character; (5) changes in osmotic pressure, though these may be regarded as special cases of chemical changes, that is, changes in concentration; (6) temperature changes.



FIG. 52.—Kymograph.
(From the catalogue of A. H. Thomas Co.)

In complex organisms, excitability does not always appear to be a response to an environmental change. The stimulus seems to arise from within. Hunger, thirst, emotions and especially the dictates of the will all seem to be inner stimuli and, in one sense, they are; yet, in the last analysis, they may all be referred to environmental stimuli, even though the responses are delayed and are complex in character.

A given type of environmental change does not always act as a stimulus. This is due to the fact that the rate at which the change occurs is a large factor in determining its effectiveness as a stimulus. This has been proved for the action of various kinds of stimuli. The action of pressure, for example, upon the frog sciatic nerve constitutes a stimulus when pressure is suddenly applied as by striking or pinching. The resulting nerve impulse causes muscular contraction. But when pressure is gradually applied to the nerve while holding it between two glass plates no response will be obtained even though pressure sufficient to permanently injure the nerve may be finally attained. Curiously enough, however, release of a pressure, insufficient to injure the tissue and so gradually attained that it does not excite, will cause a contractile response in the muscle when the pressure upon the nerve is released with sufficient suddenness. Change of pressure is thus seen to be a stimulus only when it is a rapid change.

The best demonstration of this general principle is given in experiments that employ electrical stimuli. Such stimuli show that if the change in voltage or amperage of current passing through a nerve occurs within certain limiting rates excitation results. But if the change of the current is either slower or faster than these limits, it does not constitute a stimulus. That a slowly increasing current does not necessarily constitute a stimulus is easily demonstrated. A nerve applied to electrodes, which are in circuit with a battery and a sufficiently high resistance, will not be excited by the small current which the resistance permits to pass through it. Moreover, gradual increase of the current, obtained by slowly decreasing the resistance, does not stimulate. This is true, even though the current finally attained is of such magnitude that when it is suddenly broken by interrupting the battery circuit, excitation occurs. Any constant electrical current passing steadily through a tissue, does not appear to excite it except at the time the current is made or broken. A change called electrotonus (p. 267) is produced in the tissue and this may result in excitation of nervous structures in some cases; but under most circumstances, the constant current does not cause any visible evidence of excitation. On the other hand, when changes in the electrical current proceed at a rate in excess of certain limits, no stimulus is obtained. Demonstration of this fact requires special apparatus in the form of condensers. Very brief and rapid discharges, so obtained, are not effective stimuli. Likewise, the rapid changes of a high frequency alternating

current do not stimulate. Thus it is shown that electrical stimuli are effective only when they consist of changes in current that occur within certain limiting rates.

Electrical Stimuli.—The use of electrical stimuli in physiological experiments has certain distinct advantages. Such stimuli can be accurately graduated in intensity and perfectly timed as to the moment of their application and their duration. An added advantage is the quick and complete recovery of the tissue from the effects of electrical stimuli. Induction currents are used most commonly because the control

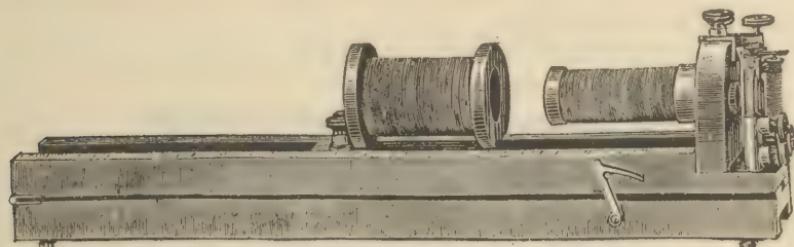


FIG. 53.—Induction coil, du Bois-Reymond form for physiological experiments.

of their intensity is especially convenient and because their duration is exceedingly brief. They may be repeated as frequently as desired without injurious results to the stimulated protoplasm. The time during which rapidly repeated induction shocks are applied is satisfactorily controlled by a suitable key in the battery (primary) circuit of the induction machine. An induction coil of the type devised by Du Bois-Reymond for use in physiological experiments is shown in Fig. 53. Works on physics must be consulted for a detailed account of the theory, construction and use of the induction coil.

Stimuli Defined.—Although the electric current has been used for experimental imitation of the natural stimuli that normally act upon plant and animal structures and although electrical stimuli probably excite in a manner that is very similar to the action of natural stimuli, yet reversible excitation has been observed, experimentally, as the result of the use of all the kinds of forces mentioned above. Such experimental results together with observations of the natural behavior of living things make impossible any hard and fast limitation of the forces that are physiological stimuli. A flexible definition of stimuli may be given, however, as any environmental change occurring at such a rate as to excite living matter. These same changes occurring at other rates are either without any effect or else produce irreversible, that is, destructive, effects upon living matter.

Even this flexible definition is not entirely satisfactory. As Loeb and others have pointed out, an environmental force, so constant as that of gravity, may constitute a stimulus in some cases. Plants respond to gravity so that their roots grow downward and their stems upward. This appears to be due to the influence of gravity in determining the relative concentrations of certain nutrients in specific parts of the plant.

Any change in the mass (concentration) of any molecules or ions that take part in a physiological reaction, may disturb an equilibrium and thereby become a stimulus. On this account a stimulus might be defined as any force which disturbs the equilibrium of any chemical or physico-chemical reaction in living matter. Constant forces, such as gravity and the constant electrical current, commonly appear to cause no excitation because, under their influence, protoplasmic reactions can come to an equilibrium.

Conduction of Excitation.—The term conduction, as applied to living matter, suggests the conduction of an electric current but should not be confused with it; for conductivity of protoplasm is a term really used in two senses. Some writers use it to refer to the conduction of an electric current through living matter in the same sense that the current is conducted through a solution of electrolytes. Other writers refer to conductivity of protoplasm as signifying the transmission of the state of being excited from one portion of a cell to another or even throughout all the cells of a tissue. This is the type of conduction here referred to and we shall specifically call it conduction of excitation. This property is commonly attributed to nerve structures as they are generally known to function in conducting that momentary state of excitation called the nerve impulse. Although nerves are especially adapted to this function and apparently are of no other use, yet, conduction of excitation is not solely characteristic of nerve but is a property common to all living matter. In other words, the mere excitation of protoplasm serves as a stimulus for other living matter provided it is in suitable, physiological connection with the initially excited region.

Cardiac Muscle.—One of the best demonstrations of this property is seen in the case of heart muscle. The normal beat of the heart involves the contraction of all its muscle fibers which act in an orderly sequence so as to produce the regular flow of blood through the heart chambers and into the arteries. This fact received its first satisfactory explanation when Bowditch (1871) showed that portions of the frog heart, quiescent because severed from the spontaneously beating portions, always responded to an effective electrical stimulus with a maximal contraction. The extent that a strip of cardiac muscle moves an attached lever is always the same under given conditions of temperature and of concentration of oxygen, inorganic ions, nutrients and fatigue products. This observation attracted attention because skeletal muscle was well known to respond with varying heights of contraction to varying strengths of artificial stimuli even though all other surrounding conditions were kept uniform. Cardiac muscle thus appeared to be unique in that its contractions were independent of the strength of stimulus. Either it entirely failed to respond or else responded with a maximal contraction. This fact was expressed by the statement that cardiac muscle acts according

to the "all or nothing" law. This law has now been shown to be applicable, in all probability, to the protoplasm of any one cell; but the "all or nothing" principle is demonstrated more easily by the use of cardiac muscle, than in experiments with other structures, merely because the cardiac muscle cells are in such closely interknit anatomical relation with one another that contractions of one cause unfailing conduction of excitation to neighboring ones. This accounts for the useful orderly sequence of contraction in the normal heart beat. Certain types of heart disease are the result of a disturbance in its normal conduction of excitation. The practical diagnosis of these disturbances has been made possible by this type of physiological study.

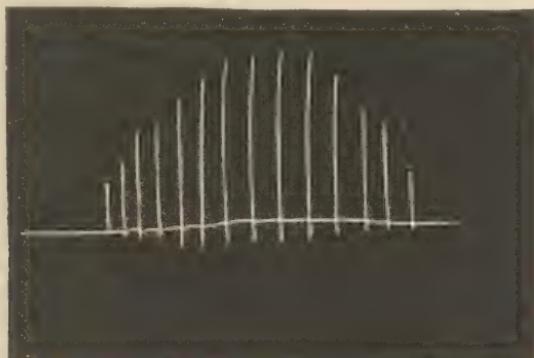


FIG. 54.—Record of the varying height of contraction of skeletal muscle with varying strength of stimulus.

Skeletal Muscle.—That skeletal muscle shows varying heights of contraction in response to varying strengths of stimulus is shown in the kymograph record of Fig. 54. The explanation of this observation is found in the varying number of muscle fibers that are excited by stimuli which vary between the minimal effective one, called the **threshold stimulus**, and the maximal one that produces the highest possible contraction. If the stimuli are applied to the nerve, its constituent nerve fibers are excited in varying numbers according to the strength of the stimulus up to the maximal. The number of muscle fibers excited and therefore the degree of shortening of the muscle is apparently determined by the number of nerve fibers excited. This seems to be the case because, as shown by Keith Lucas, the possible variations in the shortening of a muscle are not innumerable, but under conditions in which only the strength of stimulus varies, differ by definite steps. The number of these steps is not greater than the number of motor nerve fibers connected with the muscle. This suggests that each individual muscle cell either contracts maximally or not at all and, indeed, microscopic observation of single muscle cells, during stimulation with varying strengths of electric currents, bears out this idea. Pratt has obtained a graphic record of this observation by

photographing the movements of microscopic droplets of mercury lying upon the exposed surface of frog muscle. The individual skeletal muscle cell, then, contracts according to the "all or nothing" law and therefore may be regarded as able to conduct excitation throughout its substance.

To some extent, excitation appears to be conducted from cell to cell of skeletal muscle. This is shown by direct stimulation of a muscle. Stimuli applied to one end of it start a wave of contraction which successively involves muscle fibers more and more distant from the stimulated end. But this transmission may be attributable to nerve fibers within the muscle. In any case, conduction of excitation from cell to cell is much more limited in skeletal than in cardiac muscle. It may not occur, normally, at all. This limitation affords a distinct physiological advantage. It permits very nicely adjusted degrees of contraction in all skeletal muscles; so that, under the control of the nervous system, accurate movements of skilled hands, maintenance of body equilibrium and many other accurate adjustments of muscular contraction are made possible.

Nerve Fibers.—The nerve fiber not only shows a high degree of conductivity of excitation, but like the muscle fiber, transmits a maximal excitation or none at all. Adrian demonstrated this fact by ingenious experiments on muscle-nerve preparations. He measured the time required for an anaesthetic, such as alcohol or morphine, to block the progress of nerve impulses excited in the end of the nerve further away from the muscle than the portion exposed to the anaesthetic. During the time that the anaesthetic is taking effect upon the nerve, stimuli have a gradually decreasing effect upon the amount of contraction of the muscle even though uniform electrical stimuli of optimal intensity are used. This result might be taken to indicate that nerve impulses vary in intensity, but obviously does not prove it; because the effect of the anaesthetic might cause a mere progressive decrease of the number of nerve fibers able to conduct the stimulus. This, indeed, is just what Adrian proved. Two muscle-nerve preparations were simultaneously anaesthetized, one having the anaesthetic applied to a single section of nerve as indicated at A of Fig. 55, while the other received application of the anaesthetic upon two separated sections (shown at B and C), each of one half the length of the single one of the other nerve. Electrodes were applied at points 1, 2 and 3 as shown on the diagram. The result of this experiment showed that, as the anaesthetic took effect, simultaneously in the three regions, stimuli of equal intensity applied by the three electrodes ceased to produce muscular contraction at different times. Stimuli through electrode 1 disappeared first and then stimuli through electrodes 2 and 3 disappeared later and *simultaneously*. This result cannot be explained by an irrecoverable decrease in the effectiveness of stimuli passing along individual nerve fibers; for if that were the case,

the stimuli from electrodes 1 and 2 should have disappeared first and simultaneously. This would follow from the fact that the anaesthetized regions, B and C, are together equivalent in length to region A; so that the decrement in the intensity of a stimulus, which succeeded in escaping from B should be sufficient to cause it to be completely extinguished in C. But if the impulse recovers its full value after having passed through B and before reaching C, it will not be extinguished while passing through C. This was found to be actually the case. Moreover,

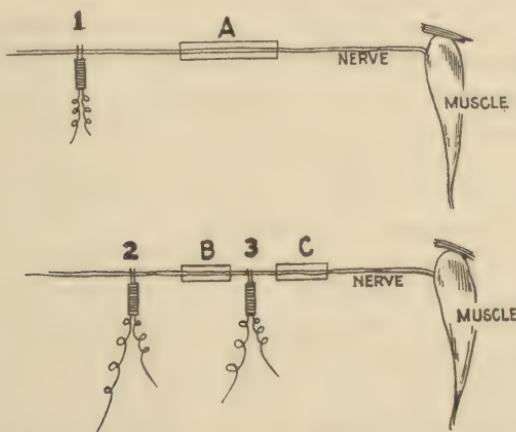


FIG. 55.—Diagram of Adrian's experiment to show the "all or nothing" behavior of nerve.
Explanation in text.

responses to stimuli, given through electrode 2, should have disappeared earlier than those given through 3 if the individual impulses showed progressive and irrecoverable decrement. But if all the impulses which enter region C are of full value, stimuli applied at 3 will be extinguished no later than those applied at 2. This was found to be the case. If, then, an impulse passes through B, it also passes through C. In other words, if the impulse passes along the normal nerve fiber at all, it goes as a maximal excitation. Although it may be decreased in intensity while traversing an anaesthetized region, it recovers its maximal value while progressing along the normal (not anaesthetized) regions.

In this sense, then, the nerve fiber conducts according to the "all or nothing" law. The only reason why the anaesthetic block upon the nerve appears gradually to diminish the effectiveness of impulses upon muscular contraction is that the number of nerve fibers which are conducting excitation from the anaesthetized region to the muscle is gradually diminished. This applicability of the "all or nothing" law to nerves is in accord with the commonly observed fact that they effectively connect mild excitation in one part of the body with extensive muscular or other response in distant portions. The "all or nothing" principle applies to any nerve fiber which is uniform in structure and is

under uniform conditions. Where uniformity is interrupted as at the anaesthetic block, excitation may be conducted "with a decrement." This is shown, in Adrian's experiment, by the fact that the longer stretch of anaesthetized nerve extinguished all impulses sooner than did the shorter stretch. Within the region affected by the anaesthetic, diminution of the individual impulses appears to be progressive. A similar progressive decrement probably occurs in certain structures even under physiological conditions. Some of the evidence for this is found in the behavior of the neuro-muscular junction.

The Neuro-muscular Junction.—A substance with excitability different from that of either muscle or nerve must exist in the neighborhood of the **motor end plates** which can be seen, histologically, at the points where the motor nerves join the muscle fibers. Evidence for the existence of this substance is found in Claude Bernard's curare experiment, described above. Some special "receptive substance" appears to be located at the junction. Its power to conduct excitation is lost under the influence of curare. Its behavior is also modified under the influence of fatigue. In advanced stages of fatigue, it does not conduct excitation so that stimulation of the nerve fails to excite the muscle although, as shown by experiments described below, both nerve fibers and muscle substance are still excitable and can conduct excitation. At a certain less advanced stage of fatigue, the neuro-muscular junction shows a peculiar phenomenon, first described by Wedensky. On applying a series of rapidly repeated stimuli to the nerve, the muscle contracts quickly in response to the first stimulus and then fails to respond further. But if the stimuli are given to the nerve at a somewhat slower rate, the muscle responds to all of them. Fatigue causes the neuro-muscular junction to fail to conduct excitations which are brought to it at intervals of a certain frequency, although it does conduct excitations which arrive at a slower rate of repetition. Lucas showed that the Wedensky phenomenon could be explained on the basis of "conduction with a decrement" at the neuro-muscular junction. It is only necessary to assume that during rapidly repeated excitation, each stimulus falls upon the nerve at a time when it is subnormally excitable as the result of the preceding excitation. This subnormal excitability, called the **refractory state**, is known to occur in muscle and nerve after all excitations. It is described below. Under these conditions, the nerve conducts a *subnormal impulse*, though without any decrement along the nerve. But in passing through the neuro-muscular junction, if it is "conducted with a decrement," this impulse might be extinguished completely before it reached the contractile structures of the muscle. But if an impulse followed the preceding one after an interval sufficient to permit the nerve to recover from its refractory state, this impulse would be of normal value and therefore might be conducted to the contractile substance of the muscle in spite

of the decrement at the neuro-muscular junction. On reaching the contractile substance, excitation would regain full effectiveness because of the "all or nothing" behavior of muscle cells. Thus, rapidly repeated stimuli would fail to excite the muscle, although less rapidly repeated ones would excite.

"Conduction with a decrement" may characterize the behavior of the neuro-muscular junction at all times, but of this there is no satisfactory evidence. The decrement appears to be more characteristic of a certain stage of fatigue. What could account for this? Increase in the C_H of a muscle is known to accompany fatigue and is probably the immediate cause of the complete failure of the neuro-muscular junction to conduct excitation in advanced stages of fatigue. The natural inference is that the partial failure of conduction of excitation, during the Wedensky phenomenon, is due to a certain C_H which, though insufficient to prevent conduction of excitation, favors "conduction with a decrement." The condition would be comparable to an incomplete anaesthesia on a portion of nerve treated as in Adrian's experiment.

The Wedensky phenomenon has attained considerable prominence in physiology because its explanation, as offered by Lucas, may apply to the conduction of excitation and the blocking of excitation at the many junctures between the functional units of the central nervous system. If this explanation does prove to have a wide application and it will doubtless be tested in future researches, it will aid in the solution of some of the most fundamental problems of excitation and inhibition. It will be referred to again in Chap. XIV.

The Fertilized Egg.—Conduction of excitation has also been demonstrated for other living structures. One more example will be given. The excitation of the egg produced by fertilization with sperm is definitely localized since the sperm is very much smaller than the egg. Yet, the effects of the entrance of one sperm spread so rapidly and completely over the surface of the entire egg as to render its surface almost instantly impenetrable to other sperm. The result is that one egg is fertilized normally by one and only one sperm even though, as in the case of most marine organisms, each egg is surrounded at the time of fertilization by a cloud of innumerable sperm.

The physiological significance of the property of living matter that enables it to conduct excitation is apparent. Correlation of activities of different portions of a cell or of the different cells of a tissue or even of whole systems of organs is made possible by physiological transmission of excitation.

Excitation of Nerves. Medullated and Non-medullated Nerves.—Nerves are commonly classified as of two types, medullated and non-medullated (Fig. 56), but this anatomical classification is not in accord with any significant differences in physiological behavior. The processes of excita-

tion and of transmission of impulses appear to be the same in these two types of nerve structure. There is supposed to be one physiological advantage in the possession of a medullated sheath. It is supposed to act as a sort of insulator to prevent the spread of nerve impulses from a medullated fiber to parallel fibers that are in contact with it. Such insulation is known to be effectively provided because impulses travelling along specific nerve fibers do not "leak out" to other nerve fibers but take effect only upon the muscle or other structure in which the specific fiber

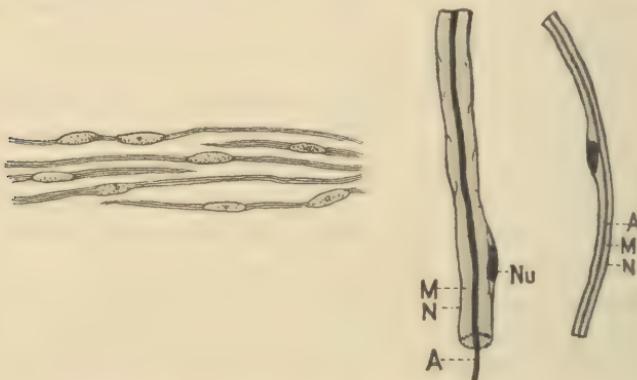


FIG. 56.—Nerve fibers, non-medullated and medullated. *A*, axon fiber of medullated nerve. *N*, neurilemma. *M*, medullary substance. *Nu*, nucleus. (After Lewis and Stohr "Textbook of Histology.")

ends. A lack of this type of insulation has never been shown, however, for non-medullated fibers so that the insulating value of the medullary sheath can be accepted only provisionally. It has been suggested that the medullary sheath may serve to insure high speed of conduction of the nerve impulse. Medullated nerves show a higher velocity of the nerve impulse.

Nerve Trunks vs. Nerve Fibers.—All types of nerves as disclosed by macroscopic dissection are really complex structures containing many different fibers. When speaking of a nerve, one ordinarily means such a nerve trunk with its many individual fibers. It is important, in physiology, to remember that the fibers composing a single nerve trunk may have very diverse connections. The vagus nerve, for example, contains fibers which connect sense receptors in the lungs, stomach and other internal organs with the brain and also contains unnumbered fibers which connect the brain with muscle fibers and gland cells in widely separated organs.

Physiological Designation of Nerve Fibers.—A classification of nerve fibers according to their functions is useful to distinguish the different fibers that occur in a single nerve. According to their functions, nerve fibers are divided into two main groups, *afferent* or *sensory* and *efferent* or *motor* fibers. The former, under most physiological circumstances, con-

vey impulses only in the direction toward the central nervous system and the latter carry them away from the central nervous system. This fact appears to be in contradiction to the general principle of conduction of excitation. It is true, indeed, that a nerve, stimulated along the central portion of its trunk, conveys impulses in both directions away from the stimulated region. In the living body, however, nerves do not normally receive stimulation anywhere except at their ends. Afferent nerves are so arranged, anatomically, that they receive their normal stimuli at their terminations in sense receptors, while motor nerves take up impulses imparted to them at their origins in the central nervous system. Accidents, such as a blow upon the ulnar nerve ("hitting the crazy bone"), are of course exceptional cases.

Nerve fibers may be further subdivided according to whether the stimuli which they conduct produce inhibition or excitation. These groups may in turn be divided according to the tissues or organs with which they are connected.

Such a classification of nerves might be supposed to imply that different nerves show different physiological behavior. One should clearly keep in mind, however, that the function of all nerves is the same: The conduction of nerve impulses. The nerve impulse is believed to be always of the same quality in all nerves. The different results obtained by stimulation of different nerves are merely due to the differences in the locations at which the impulses are delivered. This fact seems obvious enough when contrasting the effect of nerve impulses upon a muscle with their effect upon glands.

It is not so obvious, however, when one contrasts the effect of stimulating an excitatory nerve with the effect of stimulating an inhibitory nerve leading to the same organ. For example, electrical stimulation of the chorda tympani nerve of an anaesthetized dog causes relaxation (inhibition) of the muscles of the arteries of the salivary gland so that the gland swells because of its increased content of blood; but similar stimulation of certain sympathetic nerves in the neck produces the opposite effect, that is, constriction of arterial muscles (excitation) and consequent shrinking of the gland. These same opposing effects may be shown for arterial muscles in many other organs and for other structures, so that it is an established fact that both excitation and inhibition may be produced in the very same cells by stimulation of different nerves.

The case of the salivary gland is particularly interesting because experiments of Langley have shown that nerve effects upon it can be actually reversed by a reversal of the normal nerve connections. Langley's experiments were possible because of the nature of the processes of degeneration and regeneration that occur in a cut or otherwise injured nerve. Such a nerve undergoes a complete degenerative disintegration of its conducting fibers in the portion thus severed from the nerve cells

of the central nervous system or nerve ganglia. The portions of the nerve fibers that are left still connected with nerve cells suffer only a very partial degeneration in the region close to the injury and the surviving nerve stump soon begins to regenerate so that it grows out along the same path that it previously occupied until its fibers eventually restore their connections with their old terminations. Langley was able to so suture severed nerves to each other that when this regeneration occurred, fibers of the sympathetic nerve grew along the paths of the chorda tympani fibers and vice versa. After regeneration was complete, he found that stimulation of the sympathetic nerve caused vascular dilatation (inhibition) with consequent swelling of the gland, while stimulation of the chorda tympani caused vascular constriction (excitation) with shrinking of the gland. These experiments thus afford excellent evidence that the character of a response (excitation or inhibition) to impulses delivered to the same cell is dependent merely upon the behavior of the particular spot of the cell at which the impulse enters. Evidence to show that excitation and inhibition of certain other muscles are likewise dependent on the nature of nerve endings has given still further confirmation, but such observations pertain more particularly to the physiology of the specific organs concerned and will therefore be described in other connections.

In spite of this view: That nerve impulses are always of the same quality and produce different results merely because they are delivered to distinctly different nerve terminations, it is, nevertheless, convenient to name nerve fibers according to the results of the impulses they convey. For example, certain groups of the very numerous fibers of the vagus nerve are designated as follows:

	Character of results of impulses.
A. Efferent Fibers.	
1. Gastro-motor.....	Excitation of muscles of stomach.
2. Cardio-inhibitory.....	Inhibition of muscles of the heart.
3. Gastro-secretory.....	Excitation of secretion of stomach glands.
B. Afferent Fibers.	
1. Reflex cardio-inhibitory.....	Excitation of nerve centers in the brain to cause inhibition of the heart muscle.
2. Reflex vaso-dilator.....	Inhibition of nerve centers in the brain so that impulses, causing contraction of arterial walls, are decreased in number.
3. Reflex respiratory-inhibitory.....	Inhibition of nerve centers in the brain so that impulses, causing contraction of respiratory muscles, cease.

The Threshold Stimulus and Summation.—Experiments upon excitation of nerves by electrical stimuli show that a single very brief current may be too weak in intensity to produce any effect although a current of the same intensity but of slightly longer duration may be effective. The minimum current that will excite under any circumstances is called the threshold stimulus. Even a subminimal current, lasting too short a time to stimulate, becomes effective if it is passed through the nerve a second time in the same direction within an exceedingly brief interval after the first passage. This interval is about 0.0008 second. It is clear, then, that although the first passage of the current does not start a nerve impulse it so sensitizes the nerve substance that the threshold of excitation is lowered. This hypersensitive condition lasts only a very short time. The combined activity of two such rapidly repeated but separately ineffective stimuli is called summation. Although the threshold stimulus and summation in the nerve have been chiefly demonstrated upon the frog muscle-nerve preparation, there is satisfactory evidence to show that the properties upon which these phenomena depend are common to all nerves.

Electrotonus.—Just as the passage of a brief subminimal current sensitizes the nerve so as to permit summation, so the passage of a constant current, though causing no apparent excitation, does alter the physiological condition of the nerve. This altered condition is spoken of as electrotonus and the constant current producing it is called the polarizing current. The condition manifests itself in an altered excitability of the nerve. At the point where the polarizing current from a battery enters the nerve (anode), excitability of the nerve is so decreased that the threshold stimulus becomes larger than normal. At the point where the polarizing current leaves the nerve (cathode), the opposite condition prevails, so that the threshold stimulus is abnormally low, in that an unusually weak and brief current passed through a minute section of the nerve, close to the cathode of the polarizing current, will cause excitation. In the region midway between the anode and the cathode of the constant current, the nerve possesses its normal excitability. These electrotonic alterations in excitability (anodal decrease and cathodal increase) are roughly proportioned, both in amount and in the length of nerve fibers affected, to the intensity of the polarizing current. This is true at least for certain intensities during a short period after it is first sent through the nerve. The current may be of too low an intensity to cause a detectable effect or it may be of too high an intensity to permit any excitability at the anode or even at the cathode after a little time. Even a current of moderate intensity, if used as a polarizing current long enough, completely suppresses excitability at both anode and cathode.

The significance of electrotonus lies in its relationship to an explanation of the nature of nerve excitation. One should recall that living

structures conduct the electric current in essentially the same manner that a solution of electrolytes does, namely, by movements of ions. It appears, then, that the ion movements caused by a polarizing current have specific effects upon excitability. The natural inference is that excitation itself consists in some type of movement of ions. Although electrotonus has been demonstrated satisfactorily only for nerves, there are other properties to be discussed later which are common to all living matter and which indicate that excitation of any protoplasm is primarily dependent upon the movement of ions.

The Refractory Period.—If an effective stimulus applied to a nerve is followed after a very brief interval, not more than about 0.012 second, by another stimulus of the same intensity as the first, the second one produces no disturbance in the nerve. This fact was established by the use of electrical detectors for nerve impulses applied in a manner to be described presently. The brief period, during which the nerve is not excitable, that is, produces no nerve impulse in response to a stimulus, is called the refractory period. The nerve is said to be, meanwhile, in a refractory state. The fine analysis of the refractory state, made by Adrian and Lucas, showed that during the latter part of the period, excitability, as measured by the threshold stimulus, gradually returns and for a short time, about 0.015 second, after the refractory period, the nerve possesses an even heightened excitability as measured by a slightly lowered threshold stimulus. This phenomenon is due, according to Adrian, to a temporarily heightened C_H in the nerve. The refractory state indicates that a brief period is required for the restoration of ions to their original positions from which they moved during excitation and for the reversal of any other changes essential to the process.

The refractory state is not confined to the point where the excitation is first set up but occurs upon any part of a nerve just after a nerve impulse has passed through it.

Electrical Phenomena of Nerves.—Inasmuch as excitation appears to depend upon movements of electrically charged bodies, ions, an electrical disturbance is to be expected in an excited tissue. The pioneer work of demonstrating such electrical phenomena was chiefly accomplished by duBois-Reymond. Credit for his brilliant discoveries is all the greater in that he accomplished his work during the middle part of the last century, before the modern development of physical chemistry. At that time electrolytic dissociation was not even dreamed of and the nature of electrical conductivity and of polarization was not as well understood as it now is.

Electrical Detectors.—The electrical disturbances that occur in living tissues are of such small intensity that instruments for their measurement and detection must be very sensitive. Detection of electromotive forces as small as 0.05 millivolt has been necessary in certain physiological

investigations. The instruments chiefly used, especially in more recent work, are the capillary electrometer and the string galvanometer. Although works on physics should be consulted for a full description of these instruments, a brief account of their construction and of the principles which they employ will be useful here.

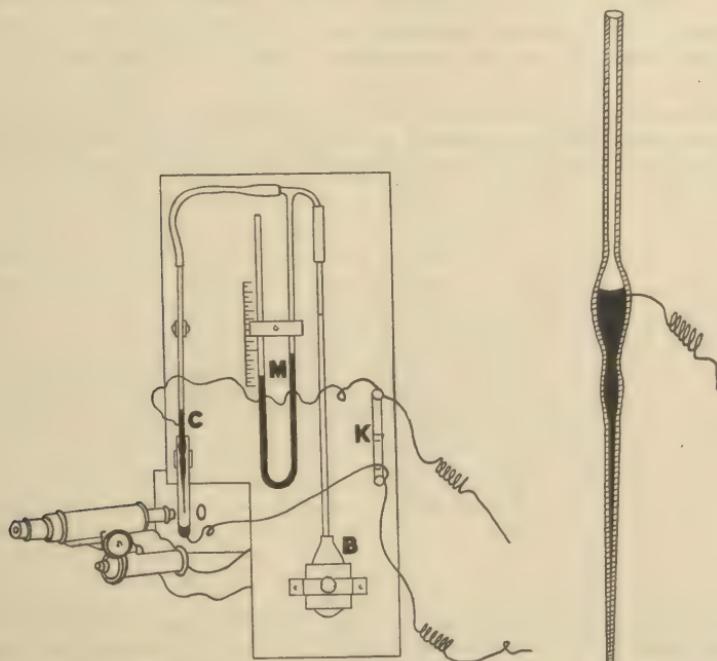


FIG. 57.—Capillary electrometer. *C*, capillary tube containing mercury and having its open lower end immersed in dilute sulphuric acid. A detail of the capillary tube is shown at the right. The lower end of the thread of mercury in the capillary is observed with a compound microscope in a horizontal position. *K*, key for short-circuiting the wires which connect the mercury and the sulphuric acid with the tissues to be tested. *B*, rubber bulb by compression of which pressure can be exerted through connecting tubes to move the mercury into the desired position. *M*, manometer which measures the amount of pressure or suction required to restore the mercury to the position it had before the passage of a given strength of current.

The capillary electrometer (Fig. 57) consists, essentially, of a capillary glass tube containing mercury in contact at the open end of the tube with dilute sulphuric acid. The mercury is prevented from running out into the acid by the force of surface tension, which in a capillary tube is sufficiently powerful to hold up a considerable weight of mercury in a vertical position. Both the mercury and the acid are connected by conducting wires to the tissue in which an electromotive force is to be detected. The meniscus at the lower end of the mercury in the capillary is observed in the field of vision of a compound microscope. As long as no electrical disturbance occurs in the circuit, the meniscus of the mercury remains at rest; but the development of potential differences in the tissue, so as to cause even a minute current to flow, so alters the surface tension of the meniscus that mercury moves. This change in surface tension is due, primarily, to the movements of ions at the mercury-acid interface, movements resulting from the passage of the current. The mercury moves down the

tube, because of diminished surface tension, when the current flows downward; it retreats upward in the tube, because of increased surface tension, when the current flows upward. The movements of the mercury can be observed through the microscope or graphically recorded upon the moving plate of a microphotographic camera. Inasmuch as the extent of the movements of the mercury meniscus, in a tube of uniform capillary bore, has a definite relationship to the potential, the voltage of a bioelectric current, as well as its direction, can be obtained by use of a capillary electrometer. This instrument has been of great service because of its surprising delicacy and low cost. It can be constructed of easily obtainable materials in any laboratory.

The string galvanometer, often called after its inventor, the Einthoven galvanometer, consists essentially of a delicate microscopic fiber of silver-coated quartz (the string) fastened vertically in a strong horizontal magnetic field between the poles of a

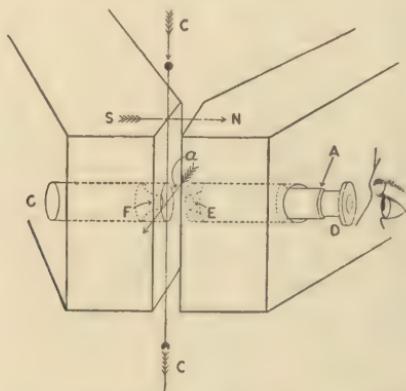


FIG. 58.—Diagram of the string galvanometer. *CC*, the string. *S* and *N*, the poles of a powerful magnet. The arrow at *A* indicates the direction of the movements of the string. *C*, source of light. *F*, objective for focussing light upon the string. *E*, objective for magnifying the string to be observed through the ocular at *D*.

powerful electromagnet (Fig. 58). The ends of the string can be put into electrical connection with conducting wires that lead to the tissue or organism in which electrical disturbances are to be detected. The principle upon which the instrument depends is that of induction which so operates that any flow of current through the silver coating of the string causes it to move at right angles to the direction of the electromagnetic field. This movement is possible because the elastic properties of quartz permit the string to bend and because the string is fastened in such a way that it can be loosened just enough to allow a certain definite amount of side-wise bending in response to the passage of a current of given intensity, for example one millivolt. The movements of the string have to be observed with a compound microscope and if they occur in the path of a beam of concentrated light, a graphic record may be obtained by the photographic method.

Other detectors, such as the electroscope and the quadrant galvanometer, have also been employed in physiology; but the two described above have proved to be the most useful ones.

Non-polarizable Electrodes.—For detection of electrical phenomena in tissues and for definitely controlled electrical excitations, it is necessary to form electrical connections between the tissues and the detectors or

the stimulators through electrodes. The development of potential differences at the contact points between tissue and electrodes, because of polarization, would create currents that interfered with detection of the bioelectric phenomena and would even excite the tissues in a manner difficult to control. It is therefore necessary in these types of physiological experiments to use non-polarizable electrodes.

Of the many patterns employed, the one shown in Fig. 59 is especially satisfactory and convenient. The clay container or "boot" is porous, thus permitting slow diffusion of solutions through it. The outer part of the clay is soaked in physiological NaCl solution and the "boot" is filled with a saturated solution of $ZnSO_4$ in which an amalgamated zinc bar is immersed. This bar is connected to the wires of the apparatus (galvanometer, induction coil, etc.). Thus any electric currents, generated by the tissue or thrown into it, are conducted by the physiological salt solution and the $ZnSO_4$ solution both of which diffuse into the porous clay of the "boot" so that they meet. But the tissue is not injured or polarized, because it is in contact with a solution containing only ions that are abundantly present in all tissues. The contact of the $ZnSO_4$ solution with the zinc bar develops no polarization. The SO_4^{2-} ions, travelling to the anode, have their electric charges neutralized by formation of $ZnSO_4$ molecules, which do not alter the composition of an already saturated $ZnSO_4$ solution; while the Zn^{2+} ions, travelling to the cathode, form molecules of metallic zinc and are deposited upon the bar.

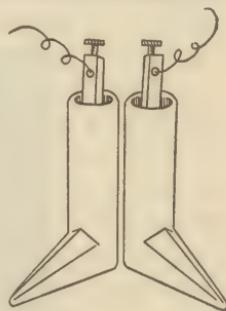


FIG. 59.—Non-polarizable electrodes. Explanation in text.

The Action Current.—If two electrodes are connected to a nerve and to an electrometer, and if one brief stimulus is given to the nerve, as indicated in Fig. 60, the electrometer shows that electrode (B), the one

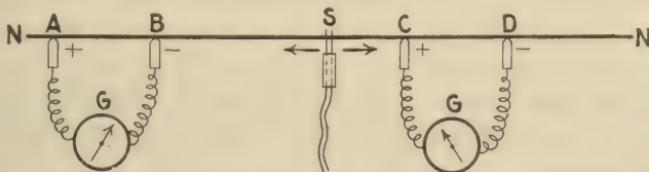


FIG. 60.—Diagram to show the relation of the nerve impulse to the wave of negative electrical variation. S , stimulating electrodes applied to the nerve, NN , which conducts the impulse in both directions from the stimulated point. A , B , C and D , non-polarizable electrodes applied to the nerve and connected to the galvanometers, G and G' . If the distance from S to B is equal to that from S to D , B will be found to be negative to A at the same moment that D is negative to C . This shows that the negative variation progresses uniformly along the nerve and that the latter quickly recovers its previous electrical state.

nearer to the point of excitation, becomes momentarily negative to the other (A) as indicated by the flow of current through the detector. But shortly afterward, the more distant electrode (A) becomes negative to (B). In other words, the electrometer shows a diphasic current flowing first from (A) to (B) and then from (B) to (A). This disturbance is called the action current of the nerve. The time required for reversal of

the current can be measured upon suitable graphic records and shows such a relationship to varied distances between the two electrodes that the phenomenon is seen to be due to the uniform passage along the nerve of a wave of electrical negativity.

Successive stimuli applied to the nerve at known intervals result in successive electrical disturbances, which, recorded by the string galvanometer, prove that one wave of negativity passes along the nerve as a result of each separate excitation. Excitation of nerve, then, causes lowering of electrical potential at the excited point and conduction of the excitation is accompanied by a corresponding transmission of this condition of lowered potential.

The Demarcation Current.—When the cut end or any fatally injured portion of a nerve is connected, through an electrometer, with an uninjured portion of the same nerve, an electric current is found to pass from the uninjured portion to the dead portion. In other words, the uninjured portion has a higher potential than the dead portion. If, on the other hand, two uninjured regions, both in a presumably normal condition, are thus connected, no potential differences are detectable in the absence of excitation. So closely does this difference of potential mark off the injured from the normal tissue that the current passing between them is called the demarcation current. It may be used to detect any traumatic areas in tissues used for experiment. It is also called the current of injury. It is even strong enough to excite the uninjured portion of the very tissue producing it. This may be shown with a freshly prepared muscle-nerve from a frog in good nutritive condition. It is only necessary to lay the nerve over a glass rod and lower the cut end of the nerve until it comes in contact with the uninjured nerve trunk. The muscle then twitches in response to the excitation thus set up in the nerve. The experiment can be performed more satisfactorily by laying the uninjured portion of the nerve against an injured portion of muscle, exposed by cutting, and then touching the cut end of the nerve against an uninjured part of the muscle.

An explanation of the cause of the demarcation current is probably found in the fact that all injured tissues show a higher conductivity for an electric current than do normal tissues. It appears, therefore, that the ions of dead tissues are comparatively free to move and that the higher potential of normal tissue, as compared with that of dead tissue, is caused by an accumulation of ions, so held as to polarize the living tissue. This idea is further substantiated by the fact that certain inorganic constituents, as for example potassium, are not free to diffuse out of normal cells, but freely pass out of dead or dying cells. The ions specifically concerned in the polarization of living cells have not been identified and the manner in which they are held has not been shown. If these facts could be established, understanding of the process of excitation would be greatly clarified.

The Velocity of the Nerve Impulse.—Inasmuch as the action current of a nerve (negative variation) consists of a wave of negative potential, travelling along with the nerve impulse, one naturally asks if this negative variation is due to the nerve impulse. To answer this question one should know whether or not the wave of negativity and the nerve impulse travel in perfect synchrony. Fortunately, the velocity of the nerve impulse can be easily determined by a method that is independent of measurements of electric variations. This method depends upon measurements of the time elapsing between the moment that a nerve is stimu-

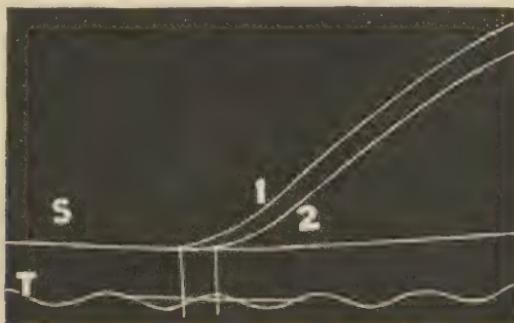


FIG. 61.—Record for measurement of the velocity of the nerve impulse. 1, the beginning of the record of contraction of frog gastrocnemius when its sciatic was stimulated near the muscle. 2, the same when the nerve was stimulated more distally. *S*, the moment of stimulating, the same for both records. *T*, the time line made by a tuning fork giving 100 vibrations per second. One complete time wave measured 29 mm. and the distance between the beginnings of the two curves measured 6.3 mm. The time required for conduction of the impulse along the nerve between the two electrodes is thus shown to be $6.3 \times \frac{1}{100} = 0.00217$ sec. The distance between the two electrodes was 64 mm. so that the velocity of the nerve impulse is computed as, $\frac{64 \times 1000}{0.00217} = 29.7$ meters per second.

lated and the moment that its muscle begins to contract. This time can be measured with a high degree of accuracy on a rapidly moving kymograph whose time record shows hundredths of a second. (Fig. 61.) This time interval is called the latent period and is found to be shorter, when the nerve is stimulated close to the muscle, than it is when the nerve is stimulated at some distance from the muscle. This difference can be due only to the time required for passage of the nerve impulse along the section of nerve between the two points of excitation, provided the same muscle-nerve preparation, the same strength of stimulus and identical conditions in all respects, save the point of nerve excitation, are employed for both measurements of the latent period. The only data required, then, for a determination of the velocity of the nerve impulse are: (1) The difference between the duration of the two latent periods and (2) the length of the section of nerve between the two points of excitation.

The actual velocity thus measured, is about 30 meters per second in the sciatic nerve of the frog at room temperature. A slightly higher velocity is found in the motor nerves of mammals. The velocity of the nerve impulse has been determined in human beings by stimulating the median nerve through the skin at two points, the elbow and the axilla, and determining the latent periods of contraction of the thumb muscles. These measurements indicate a velocity of about 120 meters per second. In non-medulated nerve fibers, conduction of the nerve impulse occurs at a much slower rate, especially in invertebrates. Velocities as low as 1 cm. per second have been observed in such nerves.

Determination of the velocity of the nerve impulse greatly helped to clarify fundamental ideas concerning the nature of excitation. Previously, the nerve impulse was not supposed to be a physico-chemical phenomenon, but was regarded in a mystical light. It was said to consist of a "psychical principle." It was supposed to move with a velocity at least as great as the passage of the electric current or the transmission of light. Johannes Müller, a leading physiologist of his time, prophesied that the velocity of the nerve impulse would never be known because a nerve of sufficient length for measurement of so great a velocity could not be found. But under the influence of a less mystical interpretation of physiological phenomena, Helmholtz made these measurements (1852) only 6 years after Müller's prophesy. The results afforded hope that even the nature of the nerve impulse and the processes of excitation which start it might eventually be understood.

One of the first fruits of knowledge of the velocity of the nerve impulse was a comparison between it and the velocity of propagation of the wave of electrical negativity in an artificially excited nerve. The two velocities are found to be identical within the limits of experimental error. The negative variation and the nerve impulse travel together and reach the muscle or other organ at the end of the nerve at the same time. The negative variation is found, moreover, to accompany impulses that are aroused by natural or physiological stimuli, for example, the sensory impulses in the optic nerve when light stimulates the retina. All sensory impulses are accompanied by action currents in the nerves that convey them and the motor impulses, starting from the central nervous system, likewise cause action currents in the motor nerves. These facts justify the conclusion that the negative variation in a stimulated nerve is actually due to the passage of the self-propagating excitation that we call the nerve impulse.

The Nature of the Nerve Impulse.—The disturbance thus passing along a nerve so as to cause a diminished potential at any point to which it has progressed, might conceivably be a physical change (such as mere movement of ions) or a chemical change (comparable to the self-propagated reaction along a fuse or train of gunpowder) or it might be a combination of physical and chemical changes. Evidence bearing on these three possibilities will now be presented.

Temperature Coefficient of Velocity.—A study of the effect of temperature on the velocity of the nerve impulse has been attempted by several investigators, notably Lucas, in order to decide whether the nerve impulse is a chemical process or a physical one. This criterion was used because van't Hoff has shown that the velocity of any strictly chemical reaction is increased so rapidly with rise of temperature that it will be at least doubled by a rise of 10°C. The ratio expressing the effect of temperature upon reaction velocities is known as the temperature coefficient. This coefficient for chemical reactions is always equal to or greater than 2 for an interval of 10°C. The corresponding coefficient for physical processes, such as conduction of an electric current, is always less than 2, although in some cases it is approximataly equal to 2. Now the velocity of the nerve impulse is just about doubled by a rise of 10°C. Its temperature coefficient is about 2. Although this result probably excludes the possibility that the nerve impulse is purely a chemical reaction, the result is inconclusive as to whether the impulse is a strictly physical process or a physico-chemical one.

Metabolism of Nerves in Relation to the Nerve Impulse.—Further evidence as to the nature of the nerve impulse has been sought in a study of the effect of the passage of impulses upon the metabolism of nerves; for if their metabolic activity is consistently increased by conducting impulses, the latter might be regarded as being themselves metabolic changes, that is, chemical reactions. One method for measuring the rate of metabolism is to determine the comparative rate of carbon dioxide production. Tashiro detected a slight increase in carbon dioxide production in stimulated nerves as compared with resting ones. He used a very delicate method capable of detecting as little as 0.0001 mgr. of CO₂. Although some metabolism as an accompaniment to nerve excitation is thus indicated its amount must be very small indeed.

Another method for measuring metabolic activity is to determine the rate of oxygen consumption. Actual measurements of this value have not been made, satisfactorily, on nerves; but indirect evidence is available, in that excitability of nerves has been shown to be dependent upon a supply of oxygen. A nerve not only loses its excitability when placed in an oxygen-free atmosphere but it even loses the power to transmit nerve impulses in any section of the nerve that is surrounded by an oxygen-free atmosphere. This loss of conducting power can be observed even when stimuli are applied to a portion of the nerve that is in air. Oxygen is necessary, then, for both excitation and its conduction. This fact clearly indicates that some sort of oxidative chemical reaction is an accompaniment of the nerve impulse. This conclusion is further substantiated by the fact that excitability and power to conduct the nerve impulse are rapidly restored to a temporarily "asphyxiated" nerve when oxygen is again supplied to it.

These observations are very difficult to reconcile, however, with the results of still a third method for the study of the rate of metabolism in nerves. This method depends upon the measurement of heat production. It has been employed by several investigators, notably Hill, who used an apparatus capable of detecting a change in temperature of 0.000006°C . But he could not detect any heat production as a result of continuous stimulation of the nerve during 25 seconds. From these observations, he was able to compute, that if a single impulse passing through the nerve produces any heat at all, it must be less than that which would be formed by the use of one molecule of oxygen by a portion of the nerve equal to $3.7 \text{ cu. } \mu$. This is equivalent to saying that the passage of the nerve impulse does not cause oxidative metabolism of the ordinary heat producing type, because the latter would certainly result in the liberation of heat sufficient to be measurable by Hill's method.

Fatigue.—Studies of the fatigue of nerves have also been used as evidence bearing upon the nature of the nerve impulse. Fatigue in most living structures, for example, muscle and the central nervous system, is known to be due to the accumulation of waste products, such as carbon dioxide, or to the depletion of available nutritive material such as glucose. If the nerve impulse, then, consists of a metabolic change, similar to that occurring in muscle, it should result in the appearance in the nerve of a state of fatigue, in which impulses are no longer excited or conducted with normal efficiency. But the curious fact is that activity does not appear to fatigue the nerve fiber at all—at least not in the ordinary sense. When the muscle nerve preparation is used to test the possibility of fatiguing a nerve, contraction of the muscle cannot be relied upon as an index of successful transmission of the nerve impulse, because the muscle itself is soon fatigued to the point of complete inactivity. But the nerve is still active. This fact can be shown by prolonged stimulation of the nerve while the resulting impulses are being prevented from reaching the muscle. This may be done in one of several ways. One way is to block the conduction of nerve impulses through a portion of the nerve near the muscle by a locally applied anaesthetic or by a polarizing constant current applied as shown in Fig. 62. Another way is to curarize the muscle. This, as explained above, blocks the passage of nerve impulses from the nerve endings into the muscle substance. With any of these methods, electrical stimulation of the nerve may be kept up continuously during ten or twelve hours, the muscle meanwhile at rest, and upon removal of the block by withdrawing the anaesthetic or interrupting the polarizing current or neutralizing the effects of curare, nerve impulses are found to be still passing along the nerve as shown by maximum contractions of the muscle. Observations of the action currents of nerves also show that nerve impulses may be made to pass through a nerve almost indefinitely without any apparent

diminution of the negative variation. Only abnormal conditions (low temperatures, lack of oxygen, presence of anaesthetics or depressants, the polarizing current, any lethal conditions) cause nerve fibers to diminish their excitability and their power to conduct excitation. Compared to other tissues, then, nerve is said to be incapable of fatigue. But in a certain sense, nerves do suffer fatigue in that they show a refractory state. As explained above, the passage of a nerve impulse or the local excitation of a nerve is immediately followed by a very brief period during which the nerve must recover from the effects or activity before it is again excitable. During the refractory period, then, the nerve may be compared to a structure that is completely fatigued; but unlike fatigue, the refractory period is of brief duration. The reactions which cause it are readily reversible, so that the nerve recovers rapidly and completely. In this respect it is comparable to the heart which throughout life maintains its rhythmic beating, resting only during the brief periods between its beats.

All these data (temperature coefficient of the velocity of the nerve impulse, results of metabolism studies upon nerves and the absence of fatigue in nerves), when taken together, suggest that the actual processes of excitation and of conduction of the nerve impulse are not dependent upon chemical reactions of the type called metabolism but are more nearly physical reactions. The data also suggest, on the other hand, that after the nerve has been excited or has conducted an impulse, some type of metabolism requiring oxygen takes place in order to restore the nerve to an excitable condition. The failure to produce any detectable amount of heat indicates, however, that this metabolism is not ordinary oxidation but is an easily reversible reaction involving oxygen. The difficulty is to reconcile this lack of heat production with Tashiro's observation upon CO_2 production, because all known reactions that use up oxygen and produce CO_2 evolve a measurable amount of heat. Tashiro's results are not accepted by all physiologists. Bayliss, for example, points out that Tashiro did not satisfactorily exclude the possibility that CO_2 , dissolved in the nerve tissue, is given off more rapidly during stimulation than it is while the nerve is resting.

Mechanical Models that Imitate the Phenomena of Nerves.—Further light upon the nature of the nerve impulse has been obtained by a study of similarly self-propagating disturbances in metallic conductors. Several

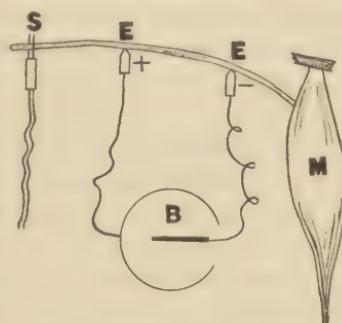


FIG. 62.—Arrangement of a polarizing current for blocking a nerve impulse. *S*, stimulating electrodes. *E*, *E*, non-polarizable electrodes applied to the nerve and connected with a battery, *B*, which delivers a constant current conducted downward, that is, toward the muscle in the nerve.

different methods have been employed in such investigations. In each case, however, the same fundamental principle has been employed: The conducting power of a metallic wire surrounded by a liquid, having lower conductivity than the metal. The typical arrangement of the apparatus is shown in Fig. 63. Such an arrangement is sometimes called a "core conductor." It is also called a "core model" of a nerve.

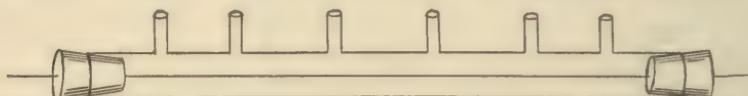


FIG. 63.—Diagram to show the arrangement of the "core model" of a nerve.

The particular type of arrangement, used by R. Lillie in his extensive investigations on this subject, is especially interesting in that it permits imitation of a larger number of the phenomena of nerve activity than do other similar arrangements. His device consists essentially of a wire of pure iron treated with a strong solution of nitric acid or with some other oxydizing agent and then placed in a weaker solution of nitric acid. Under these conditions, the wire, covered with a very thin film of oxide, is not attacked by the nitric acid but remains in a "passive" state unless it is in some way disturbed.

It now possesses, however, a high degree of what might be called sensitivity or responsiveness to the same conditions that stimulate nerves. It is sensitive to mechanical "stimuli" such as scratching with a piece of glass, and responds to this "excitation" by showing a rapid disappearance of the film of oxide in the immediate vicinity of the scratched point. This disappearance is due to a self-propagating disturbance, for it can be seen to travel from the "excited" point along the wire. The model likewise responds to electrical "stimuli" for if a suitable current is passed through a small section of the wire, it is "excited" at that section and the resulting disturbance is self-propagating. This localized "exciting" current breaks the film upon the surface of the iron wire because an electro-chemical reduction of the oxides in the film occurs. Indeed, any force, mechanical, chemical or electrical, that breaks the continuity of this surface film is sufficient to start a reaction along the surface of the wire.

The progress of the reaction is easily followed by observing the effervescence and the formation of dark oxides that accompany it. It can also be followed by the use of galvanometers connected by side wires to the "core conductor." The galvanometers are deflected in such manner as to show that any portion of the wire at which the disturbance has just arrived has a negative potential as compared with any portion that is still enfilmed. This electrical behavior is thus comparable to the action current of an excited nerve. The rate at which the disturbance is propagated along the wire is comparable to the velocity of the nerve impulse

and, like the latter, can be varied by temperature and other surrounding conditions from a few centimeters per second to a few meters per second. Moreover, the reaction which constitutes the disturbance is a reversible one, if the wire is surrounded by the correct concentration of nitric acid; so that the wire regains its film and becomes again "passive" shortly after the passing of the disturbance. Having so recovered, it is ready for another "excitation" and can be used for repeated demonstrations an indefinite number of times. It is thus comparatively "indefatigable" in somewhat the same sense as is a nerve. But like the nerve, it does show a momentary "fatigue" in that it has a "refractory period" during which "excitability" is absent. Any part of the wire that has been disturbed or any part to which the disturbance has been propagated cannot respond to a "stimulus" until that part has had time to recover its former "passive" (enfilmed) state.

The behavior of Lillie's "core model" is still further comparable to that of a nerve fiber in that the nature and intensity of the "stimuli" which "excite" the wire are similar to the corresponding qualities of nerve stimuli. The "exciting" force must attain a certain minimal (threshold) intensity to be effective. A mechanical "stimulus" (scratch) that is too slight to break the film or an electric current that is too weak or too brief does not start any disturbance in the wire. But like the nerve, the wire is changed by such a "stimulus" so that a second application of the same subminimal force may cause effective "excitation" if applied soon enough after the first one. This phenomenon is comparable to summation in the nerve. Also a constant electric current, gradually introduced, does not "stimulate" the model but acts as a polarizing current to produce lowered "sensitiveness" (heightened "threshold stimulus") at the anode. Thus the model imitates electrotonus of nerves. The constant current acts as a "block" to the passage of disturbances in the wire, as it does on the nerve.

In still another way, the model illustrates the behavior of nerves; in that the disturbance, if it passes along at all, breaks all the film upon the surface of the wire at any one point. It therefore shows the maximum effect at any given point. In this respect, it imitates the "all or nothing" principle as shown by nerves.

The value of these observations depends chiefly upon the fact that the conditions determining the behavior of the wire are essentially understood and can be described in terms of physical chemistry. These studies, therefore, give a tentative explanation of the nature of the nerve impulse in terms of physical chemistry. The conditions and processes that occur in the model and their supposed parallels in the nerve are conceived by Lillie somewhat as follows: (1) A film of uniform thickness composed of iron oxides exists upon the entire surface of the "passive" wire so as to check reaction between it and the surrounding fluid. The

corresponding condition postulated for the resting nerve is the existence of a thin, uniform film at the interface between the axon fiber and the surrounding protoplasm. Unfortunately, the existence of such a film and the chemical nature of the substance that might compose it, can only be surmised; but many facts of the physical chemistry of colloids (Chap. X) and numerous physiological observations clearly indicate the formation of such films at phase boundaries essentially like this interface. (2) "Excitation" is a localized disturbance of the film of such a character as to produce a break in its uniform continuity. A corresponding break is postulated for excitation of the nerve. (3) As a result of this break, there is a difference of potential between the "activated" ("excited") portion of the wire and the adjacent "passive" (enfilmed) portions. Lillie has detected this potential difference immediately after scratching

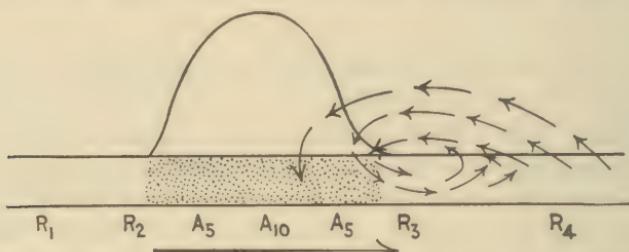


FIG. 64.—Electrical conditions as related to a self-propagated disturbance. The large arrow shows the direction of the propagation of the impulse. The shaded region is the part of the nerve that is excited at the given moment. The intensity of excitation in its different regions is indicated by the curve above. The maximum of the disturbance (excitation) is at A_{10} with a lower intensity at A_5 . Regions R_1 and R_2 are completing recovery. In regions R_3 and R_4 the potential is sufficiently above that of the excited region to set up the electrical currents represented by the curved arrows. This current excites R_3 and R_4 so that the disturbance is propagated. (After Lillie, *Physiol. Rev.*)

the wire while it was appropriately connected to a galvanometer and has found that the "excited" region is negative to adjacent ones. It is true, indeed, that any two different metallic substances that are in contact with each other and with a conducting solution show a difference of potential between them, provided they have different solution tensions. Pure iron and iron oxide have different solution tensions and therefore exhibit a difference of potential under these circumstances. The parallel condition in nerve is the rapidly developed negativity at its excited, active point. (4) A localized electric current flows from the "passive" to the "activated" portion through the surrounding solution. The corresponding process in the nerve is the current of action (the negative variation). (5) The current thus established causes a disturbance of the film in the region adjoining the previously activated one so that this new portion becomes itself activated and attains a negative potential as compared with regions still further along. Thus the whole process of "excitation" is propagated as a wave of activity. The corresponding process in the

nerve is the conduction of the nerve impulse. (6) The action of the nitric acid surrounding the wire oxidizes the iron so as to restore the film upon it. The corresponding process in the nerve is its recovery of excitability during the refractory period. These conditions and processes as conceived by Lillie are represented diagrammatically in Fig. 64.

Conclusion.—Inasmuch as the physico-chemical conditions at the interface between the axon fiber and the surrounding substance of the nerve are still unknown, an exact interpretation of nerve excitation and its propagation is still impossible; but the parallelism between nerve and model favors the inference that excitation is a disturbance of the equilibrium of conditions which, in a resting nerve, maintain the continuity of a film. This parallelism also favors the idea that conduction of excitation is due to the development of a bioelectric current that results from the difference of potential between the excited and the resting region—a current sufficient to excite the resting region involved. This conception does not clearly indicate to what extent chemical processes, as distinct from the physico-chemical ones involved in disturbance of the film and consequent development of differences of potential, are concerned in nerve activity. The conception implies, however, that just as an oxidative effect of nitric acid must be exerted upon the "excited" iron wire before it is again restored to its "passive" but "excitable" condition, so some chemical reaction is involved in the restorative processes that occur during the refractory period in the nerve. The need of oxygen for maintenance of nerve excitability favors the idea that this restoration is an oxidative process. It is probably a chemical process of some kind. The refractory period is shortened by a rise in temperature more markedly than the velocity of the nerve impulse is correspondingly increased. The processes of the restoration of nerve excitability are shown by this shortening of the refractory period to have a temperature coefficient of 3 or more for 10°C . This, as pointed out by Adrian and others, indicates that the restoration process is a chemical one. The reaction cannot be a profound one since metabolism in the nerve is very small, as shown above.

Excitation of Muscle.—The phenomena of excitation of muscle are essentially the same as those of nerves. One is accustomed, perhaps, to think of contraction as the only physiological event that occurs in muscles; but as a matter of fact, contraction is only secondary to the actual excitation process. The physiology of contraction will be considered in the next chapter. Only the actual excitation process will be discussed here.

Electrical Phenomena.—The electrical conditions of muscle tissue show many points of similarity to those of nerves. Any injured part of a muscle has a negative potential as compared with an uninjured part, so that a current of injury or demarcation current may be detected by the same

method used for nerves. A stimulated muscle also shows a current of action or negative variation. This variation has been observed in stimulated muscles, however, even when no contraction could be detected. Certain drugs act upon the hearts of the frog and the tortoise so as to make them incapable of contracting, while the electrical change, in response to artificial stimuli, is still present. This electrical change has been shown to occur in all the different kinds of muscle that have been investigated and to take place before any actual contraction begins. The process of excitation in a muscle is believed, therefore, to be entirely independent of the process of contraction and probably occurs in muscles in essentially the same manner that it does in nerves.

Conduction of Excitation.—As stated above, a wave of contraction spreads over a muscle when it is locally stimulated in a restricted portion. This demonstrates conducting power in muscles. There is also evidence to show that the power of conducting excitation is not dependent upon the process of contraction. This fact is proved by several different methods of experimentation. In one of these methods a muscle is placed so that part of it is in water. After a certain time the action of water destroys contractile power in the water-soaked portion, but leaves it still capable of conducting excitation; so that when a stimulus is applied to it, the part of the muscle not immersed in water is made to contract. Other experiments have shown similar results with heart muscle. Under certain circumstances, a portion of the heart, the auricles, for example, may fail to contract and still conduct a wave of excitation through their muscle substance so as to excite contraction in the ventricles. These and other similar experiments have made it entirely probable that conduction of excitation is entirely independent of muscular contraction.

The Latent Period.—A method for the measurement of the time interval between the moment a nerve is stimulated and the moment its muscle begins to contract, was described above. By a similar technique, it is possible to measure the time interval between the moment of a direct application of a stimulus to a muscle and the beginning of its contraction. This interval is the latent period of muscular contraction. Its duration is different in different muscles. For the gastrocnemius of a frog, the latent period is about 0.01 sec. In some types of muscles, especially the involuntary muscles, it is much longer. In all muscles, it varies widely with conditions of temperature, nutrition, etc. During the latent period, the only changes that have been detected, are those producing the electrical variation. The muscle is occupied, during the latent period, with the process of conducting the wave of excitation and with the development of the tension which eventually causes contraction.

The Refractory Period.—Muscles, like nerves, show a temporary loss of excitability during the period of recovery from a previous excitation. With most muscles, this refractory period cannot easily be demonstrated,

because all of the many fibers composing a muscle do not contract necessarily at the same time. Consequently, certain fibers are apt to be in an excitable condition and to respond to stimuli even while other fibers are refractory. But in cardiac muscle which, as explained above, gives a complete demonstration of the "all or nothing" law, all its fibers contract under normal circumstances in due order. It is, therefore, easy

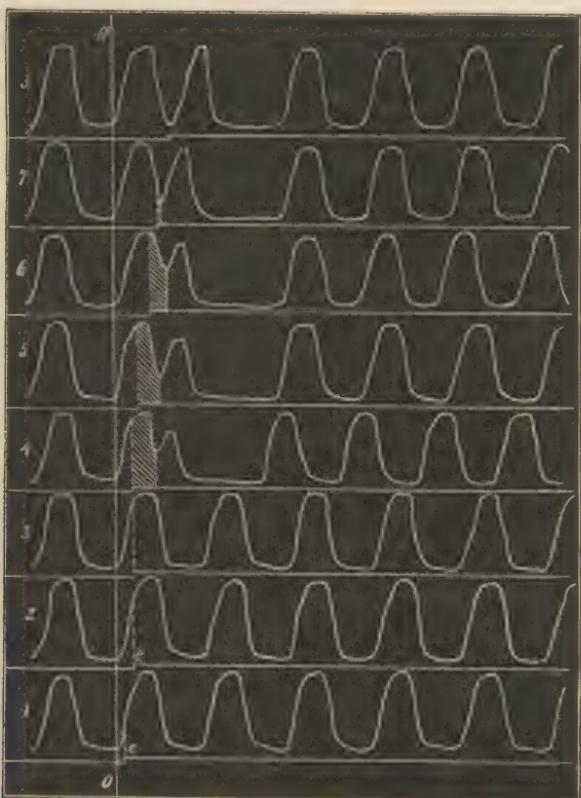


FIG. 65.—The refractory period of the heart. While the rhythmic beating of the ventricle was being recorded, electrical stimuli were applied in such a way as to fall upon the heart at different stages of its activity. The moment of stimulation is shown by the signal, *e*. The record shows that stimuli which are received during the contracting phase of the heart beat have no effect upon the heart. Stimuli which are received during the period of sustained contraction produce a small extra beat. The latent period of this effect becomes shorter and the extent of the resulting contraction greater when the stimulus occurs during the relaxation phase of the heart beat. After each extra beat the heart shows a compensatory pause before resuming its regular rhythm. (After Marey, from Howell's "Text Book of Physiology," W. B. Saunders Co.)

to demonstrate the refractory period of heart muscle. A graphic record of the effects of electrical stimulation of the ventricle is given in Fig. 65. The tracing shows that the heart is refractory throughout the greater part of the time that its fibers are shortening. A method which detects

the excitability of single muscle fibers is able to demonstrate the refractory period in any type of muscle cell.

Conclusion.—From these facts, it appears that the only fundamental difference between excitation in muscles and excitation in nerves, is that the subsequent events lead to contraction in muscle but merely to conduction of excitation in nerves. Because of the similarities between these two processes of excitation and the corresponding ones in other tissues, it is highly probable, as pointed out by Lillie and others, that any theory which correctly accounts for the nature of excitation and its conduction in one type of structure is applicable, with only slight modifications, to all protoplasmic structures.

Excitation of Glands.—Glands may be excited to secrete by two types of stimuli: (1) Impulses which enter the gland through nerves, and therefore normally come from the central nervous system, but which may be effectively produced by any of the artificial stimuli described above; (2) chemical agencies in the form of specific glandular excitants brought to the cells by the circulation.

Normally, excitation of glands by nerve impulses results from reflex stimulation. The stimulus, exciting a sensory organ, produces afferent impulses which are conveyed to the central nervous system. The impulses then excite certain connecting nervous elements and these in turn excite efferent impulses which are conducted by efferent nerve fibers to the gland. The reflex excitation is well illustrated in the control of the salivary glands. The tasting, chewing and smelling of food act upon sensory nerve endings in the mouth or nose and a flow of saliva into the mouth results. Even the thought of food, when one is hungry, causes "watering of the mouth." The stored up impressions of memory are thus able to instigate nerve impulses for excitation of glandular secretion. Such an effect is called a "psychic reflex" stimulation. These different types of reflex stimuli have been shown to excite other glands of the body in much the same way that they act upon the salivary glands. Even certain ductless glands, as for example, the adrenals, have been shown to give off their characteristic secretion into the blood under the normal control of reflex nervous excitation. A more detailed account of the nervous control of glandular activity will be given later in connection with the physiology of the several glands.

Chemical excitation of glands has not been understood as long as the nervous type. The first definite proof of the existence of the chemical type of control was given by Bayliss and Starling (1902). They showed that the pancreas, though probably not entirely free from nervous control, is excited to secrete pancreatic juice chiefly by a substance brought to it by the blood stream from the walls of the small intestine. They named this exciting substance *secretin*. Although its molecular structure is still unknown, its chemical properties and its physiological effects

upon the pancreas have been extensively investigated (Chap. XVII). Secretin is a type of numerous physiological excitants that are in each case produced in one part of the body, conveyed by the blood into the general circulation and made use of in some more or less distant part of the body as excitants of secretion or other activity. These substances are called chemical messengers or hormones (from the Greek, to arouse to activity). Their chemistry and physiology will be considered later in connection with their several functions.

Chemical excitation of glands may also be produced by drugs such as the vegetable alkaloid, pilocarpine, which is a general excitant for glands. When injected into the blood it causes copious secretion of practically all glands. It appears to excite the gland cells directly, without intervention of nerves, because it arouses secretion of mucus in glands of the respiratory passages, glands which have no nerve connections.

Excitation of Plants.—Although there are reasons for believing that the protoplasm of all plant cells is excitable, the processes, resulting from stimulation of plants, have been studied chiefly in those forms that show comparatively rapid movement—the sensitive plant (*Mimosa*) and the fly-trap (*Dionaea*). In the latter, rapid movements occur when a fly or other object touches certain sensitive hairs on the upper surface of the leaf. The leaf then shuts up quickly. Artificial stimuli produce this same reaction and the same types of electrical, mechanical and other stimuli that are used in animal experimentation, are effective upon this and similar plants. The processes of excitation and conduction appear to have fundamentally the same characteristics in these plants, that they have in animals. There is the same action current which, if detected by two electrodes leading off to a galvanometer, shows that the electrode nearer to an excited spot, is first negative to the other electrode, while a moment later the further electrode is negative to the nearer one. In other words, these plants have a wave of negative potential, sweeping over them as an accompaniment of the spread of excitation. These plants also show threshold stimulus, summation and a refractory period in close correspondence to the same phenomena in animals.

All green plants are capable of responding, by bending, to the stimulus of light and this stimulus is strong enough to overcome gravity and often-times other pressures. The excitation by light can be transmitted through the plant, without being dependent upon the bending response. These and many other interesting, detailed observations upon plant behavior have led to the general conviction that plant protoplasm is excitable in the same sense that animal cells are and that the excitation processes are essentially the same in plants and animals. The only reason why we do not see quick and sensitive responses in plants to diverse stimuli is that plant protoplasm is commonly provided with a hard non-living, insensitive cell wall and does not possess highly sensitized surfaces

such as occur in sense organs, nerve cells, nerve fibers, muscles, glands, etc. Moreover the plant is not provided with a special system for conducting excitation, comparable to the nervous system. In most plants, the excitable structures are comparatively isolated and buried deep in the cells, where they cannot be stimulated effectively.

Views as to the General Nature of Protoplasmic Sensitiveness.—Excitation appears to be fundamentally a disturbance of equilibrium in thin films and appears to involve ion movements which produce bioelectric currents. This idea is of course very incomplete in that it does not even attempt to account for the nature of the primary disturbance in the excitable protoplasmic film nor does it attempt to state what might be the nature of the effects of excitation upon parts of protoplasm adjacent to the excited films. Several different concepts have attempted to fill in these gaps. One of them is the idea of a change in the condition of an emulsion (reversion), a concept outlined in Chap. III. The limitations of this theory were stated there. Another concept suggests that protoplasm is excitable because its proteins, under the influence of movements of hydrogen ions with resulting localized changes in concentration of hydrogen ions, can change their combining powers and their attraction for water molecules. This theory was outlined in Chap. X. Neither of these theories is entirely adequate to fit all cases. It is possible that each is a partial aspect of a complete truth.

Inhibition of Heart Muscle.—Living matter is probably as generally capable of showing inhibition as it is of showing excitation. Conditions for normal production of inhibition are not as common, however, as are those favoring excitation. The most thoroughly studied and longest known case of inhibition is that of the heart. Since 1845, it has been known that stimulation of the vagus nerve causes a stopping of the heart beats or a decrease of their rate and force. Although the cardiac muscle maintains its rhythmic contractions automatically, the contractions are nevertheless responsive to outside influences, chief of which are nerve impulses that normally come from the central nervous system via two sets of nerve fibers. One set lies in certain nerves of the sympathetic system and excites cardiac muscle to accelerate or augment its beats. The action of these nerves will be described in connection with the physiology of the heart. The other set of fibers lie in the vagus nerves and connect with the heart through the cardiac branches of the vagi. Impulses, reaching the heart via these fibers, cause inhibition. A tracing, made by attaching a lever to the exposed heart of a frog, is shown in Fig. 66. After cutting the vagus nerve near the base of the skull the end connected with the heart was stimulated electrically by induction shocks. The tracing shows that the stimulus caused complete stoppage of the heart after a certain latent period. By graduating the strength of the electrical stimulus the effects on the heart can be correspondingly

graduated, so that either the rate or the force of the beats or both rate and force are decreased without complete stoppage (Fig. 67). In some experiments, the heart is stopped completely but soon resumes its beating in spite of continuation of vagus stimulation. This phenomenon is called "escape" from the vagus. Different species of animals vary in their heart response to vagus stimulation. In cats, for example, it is almost impossible, even with maximal stimuli, to stop the heart completely and if it does stop, it soon "escapes." In dogs, on the other hand,

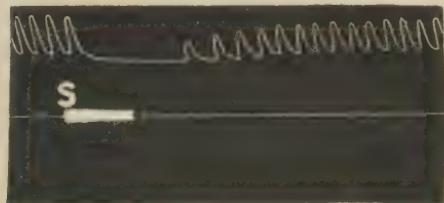


FIG. 66.

FIG. 66.—Inhibition of the frog heart by the vagus. The vagus was stimulated during the time indicated at *S*. After a short latent period the heart was completely stopped and remained quiescent for a short time after stimulation ceased.

FIG. 67.—Incomplete inhibition of the heart by the vagus. Stimulation lasted during the period indicated at *S*. The heart shows escape from the vagus. It is slowed but not stopped.

mild stimulation of the vagus markedly affects the heart and in some cases complete stoppage of the heart has been maintained long enough to kill the dog, although the heart is found to be able to "escape" from inhibition in most dogs. Apparently, different individuals of the same species vary considerably in this respect. In certain species of cold-blooded animals the heart may be inhibited for a long time. In the tortoise, for example, the heart has been held in a completely relaxed state during four hours by continuous electric stimulation of the vagus. Inasmuch as life in the tissues of these animals is not dependent upon a continuous supply of oxygen from steadily circulating blood, the tortoise heart can resume normal beating even after this prolonged period of arrest. To some extent, the individual and species variations in cardiac inhibition are attributed to variations in the anatomical disposition of inhibitory nerve fibers. In some animals none of these fibers occur in one vagus though experiment demonstrates their presence in the other. The proportion of fibers in the vagi appears to vary considerably in different individuals of the same species.

Reflex Inhibition of the Heart.—Inhibition of the heart can be obtained by excitation of structures other than the vagus. One of the earliest demonstrations of this fact was given by the Goltz tapping experiment. In this procedure, a succession of rapid, light taps upon the skin of the

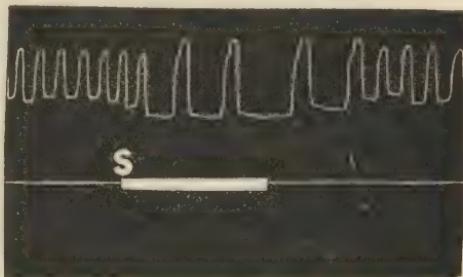


FIG. 67.

abdomen of a frog causes a slowing or even temporary stoppage of the heart. This result fails, if both vagus nerves of the frog have been previously cut. The effect is therefore exerted upon the heart through the medium of the vagus nerves. Similar observations are frequently made upon all sorts of laboratory animals and show that stimulation of almost any sensory nerve is liable to result in inhibition of the heart. Stimulation of the central stumps of severed nerves is the usual method for obtaining this result. Certain nerves when thus stimulated are particularly apt to cause this effect and are accordingly spoken of as reflex inhibitors for the heart. Curiously enough, the vagus nerve itself is particularly effective in this respect. If one vagus of an anaesthetized animal is cut and its central stump stimulated, the heart is inhibited. All such indirect inhibitions of the heart disappear if both vagus nerves are cut.

These indirect inhibitions occur in human beings. Severe pain in any one of several parts of the body, especially in the testes, may cause a marked temporary slowing of the heart. A sudden intense pressure upon the eyeball will sometimes produce the same effect. This procedure has been used as a practical means of checking the disease symptom, called tachycardia, a very rapid heart rate. That these effects are exerted through the vagus is shown by the fact that direct stimulation of the vagus nerve in human beings—a stimulation obtained by suitable pressure upon the neck, can inhibit the heart.

All these indirect stimuli act through the central nervous system to give a reflex effect. The particular region of the brain concerned in the reflex has been approximately located by direct electrical stimulation of the brain. It lies in the part of the medulla from which the vagus nerves take their origin. Although histological researches have not satisfactorily traced the inhibiting fibers back to the particular brain cells with which they are connected, the region is, nevertheless, located with sufficient definiteness to warrant the assumption that it consists of a certain limited group of cells. They are called collectively the **cardio-inhibitory center**. Sensory impulses play upon this center to excite its cells. The resulting nerve impulses go out along certain vagus fibers to inhibit the heart.

Effect of Cutting the Vagi.—When the vagus nerves are cut, the heart beats faster. It usually shows only a slight increase upon cutting of one vagus and a larger effect when the second vagus is severed. The faster rate causes a rise of blood pressure in the arteries owing to the increased volume of blood pumped out of the heart. This result is shown in Fig. 68. Cutting the vagi is thus shown to release the heart from inhibitory stimuli previously coming from the central nervous system. This indicates that afferent stimuli play upon the cardio-inhibitory center to keep it mildly excited. It is supposed that these

stimuli come at sufficiently frequent intervals to keep the center excited throughout life. This idea is expressed by the statement that the vagus always keep a "brake" on the heart. The origins of the afferent impulses, thus acting through the cardio-inhibitory center, are not all known but one that has been particularly studied is the sensory apparatus of the heart itself. As stated above, stimulation of the central stump of the cut vagus (the other vagus being intact) is particularly apt to inhibit



FIG. 68.—The effect of cutting the vagus nerves. The two vagus nerves were severed, respectively, at 1 and 2. The resulting rises in blood pressure, measured by comparison with the scale, were due to increases in the heart rate. The small waves on the blood pressure curve represent the individual heart beats. (After Dawson, from Howell's "Text Book of Physiology," W. B. Saunders Co.)

the heart. Experiments, chiefly upon rabbits, have shown that one particular branch of the vagus is provided, noticeably, with fibers whose stimulation causes reflex inhibition of the heart. This branch is known as the depressor nerve. It is apparently provided with afferent fibers. After it is cut, stimulation of the distal stump produces no effects. Stimulation of the central stump causes inhibition of the heart and other reflex effects. The endings of this nerve are in certain parts of the heart and in the walls of the aorta. These endings are excited at every heart beat. Einthoven has shown, with the string galvanometer, that an action current passes along this nerve and up through the vagus in synchrony with every heart-beat. (See Fig. 164.) It is supposed that the blood pressure produced by each contraction of the heart, or possibly

some other force, excites the sensory endings of the depressor nerve. The entire apparatus, consisting of sensory endings, afferent fibers, cardio-inhibitory center, efferent inhibitory fibers and cardio-inhibitory nerve endings may be likened to an automatic governor on a steam engine, for the faster the heart beats, the more it would tend to inhibit itself. It is undoubtedly true, however, that the sensory impulses, capable of affecting the inhibitory center are numerous and diverse.

Inhibition of Inhibition.—Any circumstance that checks the sustained activity of the cardio-inhibitory center permits the heart to beat faster. Hunt has shown that when all the accelerator nerves of the heart are cut, stimulation of certain sensory nerves causes a reflex hastening of the heart, although this effect is abolished if the vagus nerves are previously severed. The best interpretation of these observations is that they prove the possibility of inhibiting the activity of the inhibitory center. Many other physiological phenomena, to be described in later pages, substantiate this interpretation. It is now generally believed that inhibitory nerve centers can be inhibited as well as excited. This belief does not require, as a necessary assumption, that the nerve cells of the brain are actually inhibited in the same sense that the cells of heart tissue are inhibited. The brain cells may be merely prevented from being excited. This idea will be developed in more detail in Chap. XIV. Whatever the mechanism may be, many nerve centers show evidence of being inhibited in the sense that they cease to send out impulses and there seems to be no reason to suppose that an inhibitory center should be an exception in this respect. The idea of inhibition of inhibition has been given some prominence in physiology and psychology. It has helped to clarify certain phenomena otherwise difficult to explain. The effect of alcohol, for example, is generally depressant, but is stimulating apparently in its psychic effects in initial stages of intoxication. This apparent stimulation is now interpreted as a depressant action of alcohol upon these centers in the brain which are ordinarily concerned with an inhibition of certain other centers.

Inhibition of Non-striated Muscle.—The involuntary muscles are those in the digestive organs, the blood vessels, certain parts of the genito-urinary system, the iris of the eye and a few other restricted locations. They are called non-striated or plain muscle because their microscopic structure does not show the banding or cross-striations that are characteristic of skeletal and cardiac muscle. Non-striated muscle is, in general, innervated by the sympathetic system. These muscles, like cardiac muscle, are capable of automatic activity, which shows itself partly in the form of rhythmic contractions and partly as a sustained condition of contraction, called *tonus*. They are also like cardiac muscle in that their automatic activities are subject to regulating influences exerted by nerve impulses. These are both excitatory and inhibitory. The excitation

phenomena are similar to those of other muscles and therefore will not be considered here but will be taken up later in connection with the physiology of the several organs that contain this type of muscle. Inhibition of non-striated muscles is illustrated by the following examples.

Inhibition of Stomach and Intestinal Muscles.—Contractions of muscles of the digestive organs can be observed in several ways. The opened abdomen of an anaesthetized dog or cat can be filled with Tyrode solution (p. 152) at body temperature and portions of the intestine or stomach fastened in a suitable position, without disturbing the contractile activities of their muscles except during the brief operative procedures.

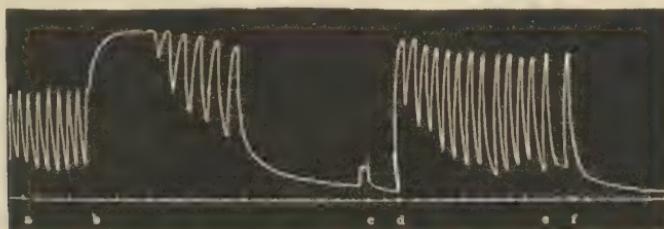


FIG. 69.—Inhibition of the intestine. Between *a* and *b* on the record, the intestine contracts rhythmically. Between *b* and *c* and at *f* the intestine is inhibited wholly or in part through the action of adrenine upon the nerve endings of the intestine. In the absence of adrenine, between *d* and *e*, the intestine resumes its beats. (From Cannon's "Bodily Changes in Pain, Hunger, Fear and Rage," D. Appleton & Co.)

If these organs are then attached to suitable levers, a graphic record of contractions and relaxations may be made. Another type of method employs photographic records of the movements of these organs by taking frequent X-ray pictures, after these organs have been made comparatively opaque to X-rays by feeding the animal a meal containing the harmless salt, subnitrate of bismuth. If the first method is used the splanchnic nerves can be stimulated electrically during observations upon the intestine. This causes a complete stoppage of the rhythmic intestinal contractions. Numerous branches of sympathetic nerves connect the splanchnic ganglion with nearly all parts of the gastrointestinal canal. The action of these nerves upon the muscles of the digestive organs is inhibitory. They do not appear to exert this influence continuously, thus standing in contrast to the effect of the vagus upon the heart; but they do exert their inhibitory effect as a result of reflex excitation. This fact has been clearly shown by X-ray observations on the intestines of cats and dogs when irritated or frightened. Such emotional states also cause inhibition of the muscles of the digestive organs in a more round-about way. Cannon has shown that emotional states cause nervous stimulation of the adrenal glands so that their secretion of adrenine into the blood is increased. Adrenine is a very powerful excitant of some substance or structure lying at the ending of sympa-

thetic fibers. As commonly stated, adrenine stimulates sympathetic nerve endings. Nervous excitation of the adrenal glands accordingly causes excitation of the nerve endings of the splanchnics and causes the same inhibition of non-striated muscle in the stomach and intestine that would result from stimulation of the splanchnic nerves (Fig. 69). Probably, both these agencies inhibit the non-striated muscles under certain circumstances. Inhibition of the stomach and intestine has been shown by Cannon in experiments on cats. While being observed with X-rays, a cat attacked by her natural enemy, the dog, exhibits a sudden and complete stoppage of gastro-intestinal contractions at the same time that her emotional state is shown by her arched back and bristling tail.

Inhibition of Arterial Muscles.—Inhibition of the muscles in the walls of arteries as a result of nerve stimulation occurs in a few instances although such inhibitions do not occur in the majority of arteries. Inhibition, when it occurs, causes relaxation or loss of tonus in these ring-shaped muscles so that the arteries dilate. Clearly proved cases of this sort of inhibition are: (1) Relaxation of arteries of the tongue during stimulation of the lingual nerve, (2) relaxation of the arteries of the salivary glands during stimulation of the chorda tympani nerve, (3) marked relaxation of the arteries which supply the erectile tissue of the penis during stimulation of the nervi erigentes.

Dilatation of most of the arteries is due to a mere decrease or cessation of the nervous impulses which ordinarily keep arterial muscles in a state of partial constriction. The actual inhibition occurs in the nervous system and not in the arterial muscles.

Reflex Inhibition of Non-striated Muscle.—Just as inhibition of the heart occurs normally as a result of reflex effects, so the inhibition of non-striated muscle is controlled through the central nervous system. Inhibition of the muscles of the digestive system and of the arteries mentioned above is the result of reflex effects under normal conditions. In contrast to the cardio-inhibitory reflexes, those which cause inhibition of non-striated muscle do not appear to be centered in a definitely localized region which would correspond to the cardio-inhibitory center in the medulla. At any rate, attempts to locate a definite center for dilatation of arteries, inhibition of intestinal muscle, etc. have not been successful. This suggests that these reflex impulses are conducted through the central nervous system by way of nervous paths that do not tend to converge at common points.

Inhibition of the Claw Muscles of the Crayfish.—An especially unique case of inhibition is found in the behavior of the claw muscles of the crayfish. The claw is provided with two muscles. They are of the striated type. Contraction of one of them closes the claw or pincers, while contraction of the other opens it. As they are thus opposed to

each other in their action, they are called antagonistic muscles. The one causing closure is much more powerful than the one which opens the pincers. The latter would be ineffective unless the closing muscle relaxed. But when the nerve of the pincers is artificially stimulated in such a way (weak induction shocks) as to cause opening, the closing muscle simultaneously relaxes. It is inhibited. Correspondingly a stimulus (strong induction shocks) which, when applied to the nerve, causes closure, inhibits the opening muscle. Biedermann and his co-workers have found that two kinds of nerve fibers enter, side by side, into each cell of these muscles. The behavior of the muscles suggests that excitation of one kind of nerve fiber causes muscular contraction and excitation of the other kind causes inhibition. The working arrangement of the nerve fibers has not been shown completely. The difficulty is that the excitatory and inhibitory fibers are contained in the same nerve trunks. There are two possibilities. Either the same fiber branches, sending one branch to excite the one muscle and another branch to inhibit the antagonist, or else there are four complete sets of nerve fibers, excitatory and inhibitory for each of the two muscles. In any case, the nerve fibers which excite the opening muscle and inhibit the closing one are responsive to weaker stimuli (applied to the nerve trunk) than are the other nerve fibers.

The instructive point, gained from a study of the crayfish pincers, is that a striated muscle can have a dual control by excitatory and inhibitory nerves. Further investigations have shown that this condition generally prevails among the arthropods.

Inhibition of Nerve Cells as the Cause of Apparent Inhibition of Skeletal Muscles.—The above illustration of inhibition in the case of muscles of the crayfish showed how inhibition of muscles could play a very useful role by preventing opposing muscles from antagonizing each other. A moment's reflection will suggest that a similar coordinating mechanism is required for vertebrates because their skeletal muscles are arranged, to a very large extent, in opposing groups. Flexors, for example, are opposed to extensors. It is necessary at times that both of these shall contract together, as in stiffening of the limbs, standing erect, etc. At times it is necessary that flexors should be inhibited while extensors contract or vice versa. An accurate dual control must exist somewhere but all efforts fail in attempts to find efferent nerve fibers, which, upon stimulation, cause inhibition of voluntary muscles. Experiments of Sherrington, Bayliss and others have shown that the real control lies in the central nervous system. In such experiments, stimulation of a set of *afferent fibers* by electrodes applied to a sensory nerve causes reflex contraction of a certain group of muscles, for example, flexors of the foot; but when some other properly chosen sensory nerve is stimulated, simultaneously, the contraction is inhibited, partially or completely, according to the comparative strengths of the two stimulations. A graphic record of such

an experiment is shown in Fig. 70. Inasmuch as such inhibitions are obtained by reflex stimulation acting upon reflex excitation, the inference is that the inhibition actually occurs only in the central nervous system. In some manner, inhibition of nerve cells, that would otherwise send out motor impulses, is produced by the inhibiting sensory stimuli. No doubt such inhibitions play a large part in the normal activities of the

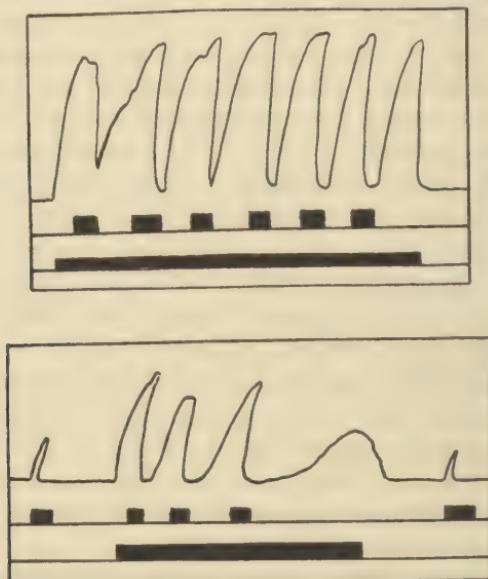


FIG. 70.—Inhibition of spinal reflexes in the frog. All sensory roots of the sciatic nerve cut. Upper tracing, contractions of the gastrocnemius muscle. Lower signal, continuous stimulation of the central end of the 9th dorsal root. Upper signal, intermittent stimulation of the central end of the 8th dorsal root. Lower tracing, similar experiment in which the action of the 10th root is inhibited by stimulation of the 8th root. The first and last contractions on the record show that stimulation of the 8th root alone causes reflex contraction, although brief. (After Vészi, from Bayliss's "General Physiology," Longmans, Green & Co.)

higher animals. It is well known, indeed, that any of the skeletal muscles may be inhibited and that the nicely adjusted contractions involved in all skillful motions and locomotions require inhibitions as well as excitations. The failure of all efforts to detect direct inhibitory action of nerves upon vertebrate skeletal muscles indicates that their inhibition must really take place in the cells of the central nervous system which controls normal excitation of the muscles.

Inhibition of Glands.—Inhibition of glands undoubtedly occurs. A gland that is actually secreting may be suddenly checked in the midst of its activity. Rapid drying of the mouth, owing to a stoppage of the secretion of saliva has been experienced, at least occasionally, by everyone. The circumstances under which it occurs are usually those of suspense,

embarrassment, or anxiety. The glass of water, so frequently placed on the speaker's desk in a lecture hall, is a frank recognition of this particular inhibition. It is sometimes said that certain of the ancient "ordeals" were based upon the same recognition. These "ordeals" were the bread and cheese test of Alexandria and the rice test of India. An accused person on trial was given a certain amount of dry food, dedicated for the "ordeal" with impressive ceremony. If he was able to chew the food with a proper flow of saliva he was judged innocent. A failure to manage the food was taken to indicate his guilt. If such "ordeals" did truly further the ends of justice, their success must have been due to an inhibition of the salivary glands by a guilty fear of detection. That the originators of these ancient "ordeals" had somehow recognized this physiological principle, is not at all impossible. Although inhibition of the salivary glands is more familiar than are other instances of the kind, suitable experiments have shown that the gastric glands and probably some others are subject to the same sort of inhibition.

Glandular inhibition is probably only inhibition of exciting nerve cells, thus comparable to inhibition of skeletal muscles. No clearly proved case of stoppage of secretion by stimulation of an efferent nerve has ever been found.

The General Nature of Inhibition.—Inhibition appears to be the reverse process of excitation. This appearance becomes almost certainty in the light of the fact that the electrical variation of the heart, when held still by stimulation of the vagus nerve, is just the opposite from what it would be if it were beating. A galvanometer connected to the beating heart shows waves of negative variation, such as are produced by all contracting muscle. But while the vagus is being stimulated, so as to hold the heart still, the galvanometer shows a positive variation. An entirely satisfactory explanation of inhibition cannot be obtained until more is known about the nature of excitation. One fact, however, is well established. If a given muscle cell is both excited and inhibited by nerve impulses, the exciting and inhibiting impulses reach the cell via different paths. The difference between excitation and inhibition is thus attributable to differences in the behavior of those portions of the cell in which the excitatory and the inhibitory nerves end, because all available evidence points to the conclusion that nerve impulses are always of the same character, no matter what nerve fibers conduct them.

A partial explanation, developed chiefly by Langley, assumes that the parts of the muscle cell which receive, respectively, excitatory and inhibitory stimuli are differently constituted. They contain presumably different "**receptive substances**" which cause opposite effects upon protoplasm under the influence of stimuli applied to the definitely localized receptive points.

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CHAPTER XII

PHYSIOLOGY OF CONTRACTION

Contractility is commonly regarded as a property of muscles but not recognized as common to all protoplasm. From the previous discussion, regarding the motility of living matter, it should be clear, however, that all protoplasm is capable of changing its shape. All the so-called contractions, even muscular contraction, are really changes of shape and are not contractions in the strict sense of that word. A contracting muscle does not decrease in volume; it merely shortens its length and increases its girth. Contractions, in the sense of change of shape, are possible in all protoplasmic structures, but occur more especially in certain structures whose form is differentiated so as to favor motility. Contractions of such specialized protoplasm are recognized as of three types: Muscular contraction, amoeboid motion and ciliary motion. The following pages will be devoted to the phenomena of these three; the physics and chemistry of each will be discussed in turn.

Structure of Muscles.—An explanation of muscular contraction is so closely dependent upon a knowledge of the intimate structure of muscles that a brief resumé of their histology will be convenient at this point. The three main types of muscles, skeletal, visceral, and cardiac, are sufficiently different in structure and their diversity of physiological behavior sufficiently dependent upon structural differences, as to make advisable a separate description of each type.

Skeletal Muscle.—This type is also called voluntary muscle (in that it commonly acts under conscious control), or cross-striated muscle because of the horizontal bandings, which characterize its microscopic appearance. A single muscle is, histologically, a complex organ composed of many cells, held together by connective tissue fibers and encased in a tough connective tissue sheath. Blood vessels in a fine and intricate network ramify the entire muscle, so that every muscle cell comes into close contact with blood capillaries. Lymph, produced by the capillaries, bathes the outside of every muscle cell. Nerve fibers constitute another intricate ramification so that nearly every muscle cell is the seat of nerve endings of one or more nerve fiber.

The muscle cells themselves are long and spindle shaped. They are commonly called muscle fibers. Owing to their great length, in comparison to their diameter, only a small portion of any one muscle cell is seen in the field of the microscope at one time (Fig. 71). The cell wall, called

the **sarcolemma**, is a definitely visible and permanent structure. Just underneath it, numerous very small nuclei are found, scattered along the entire length of the cell. Fine micro-dissection reveals the presence of numerous minute **fibrils** (**fibrillae**), which are packed closely together and



FIG. 71.—Skeletal muscle. Shows the ends of muscle fibers as they pass through transition zones into tendon. (After Lewis and Stohr's "Text Book of Histology.")

run lengthwise through the entire cell. A small amount of fluid material, the **sarcoplasm**, surrounds the fibrillae. Alternate light and dark bands occur in the fibrillae and since these bands lie in corresponding horizontal positions, they give the appearance of cross-striations to the entire muscle.

The banded appearance is due to the fact that the materials in the light and dark bands have different optical properties, refracting light to a different degree. This difference is still further emphasized when the muscle is viewed microscopically in polarized light.



FIG. 72.—Visceral muscle cells. From the small intestine of a frog. (From Lewis and Stohr's "Text Book of Histology.")

Visceral Muscles.—This type of muscle is also known as involuntary muscle or as non-striated or smooth muscle.

The cells are in the shape of short spindles with pointed ends and possess comparatively large single nuclei in the middle of the cell. (Fig. 72.) As in skeletal muscles, the cells are arranged in closely compacted form, are held in place by connective tissue fibers and are penetrated by a network of blood vessels and nerves. The fibrillae of the individual cells are difficult to make out. Sarcoplasm is found in these cells.

Cardiac Muscle.—The muscles of the heart are characterized by irregularly shaped cells of a somewhat branched and truncated outline. (Fig. 73.) Cross-striations are present as in skeletal muscle, but the nuclei and the arrangement of cells are more like that seen in visceral muscles. Corresponding to these structural distinctions, cardiac muscle is, in a way, intermediate in its behavior between that of skeletal muscle and of visceral muscle.



FIG. 73.—Cardiac muscle cells.

Significance of Differences in Muscle Structure.—The differences in structure as seen in these three types of muscle correspond to differences in their physiological behavior. Skeletal muscles contract most rapidly, visceral muscles least rapidly and cardiac muscles at a rate intermediate between those of the other two types. Skeletal muscles show very little inherent capacity for the type of sustained contractile activity called tonus, visceral muscles show this activity to a high degree and cardiac muscles are intermediate in their tendency to show tonus. Skeletal muscles are not especially efficient in the process of conduction of excitation from cell to cell, while visceral and cardiac muscles carry out this process very effectively.

The Simple Contraction or Twitch of Skeletal Muscle.—When a muscle contracts, in response to a single stimulus such as is obtained by the making or breaking of an electric current, the muscle response is called a simple contraction or a twitch. The methods for preparation of the muscle-nerve for excitation with induction shocks and for graphically

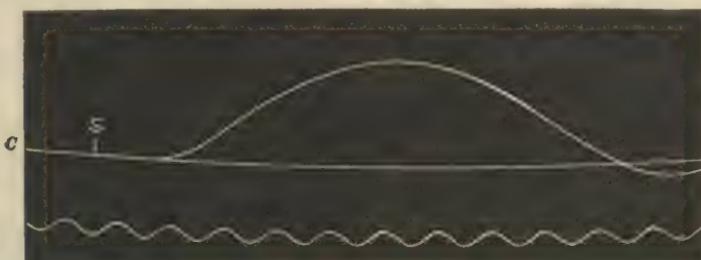


FIG. 74.—Record of simple contraction of muscle. *S*, the moment of stimulation. Reference to the time line (one complete wave represents 0.01 sec.) permits measurement of the duration of the latent period, the phase of shortening and the phase of relaxation. (From Howell's "Text Book of Physiology," W. B. Saunders Co.)

recording the contraction were given above. By reference to Fig. 74, a typical record of a simple contraction, the curve is seen to reveal the duration of three periods: The latent period, the contraction period (phase of muscle shortening) and the relaxation period (phase of muscle restoration). The first of these three periods is approximately 0.01 sec. Its duration varies in accordance with the kind of animal, the temperature and other conditions of the muscles and of course is greatly exaggerated by any "lost motion" in the recording apparatus. The second phase, contraction, lasts about 0.04 sec. in frog muscle under ordinary laboratory conditions and the third phase, relaxation, about 0.05 sec. The total time of the simple contraction is, then, about 0.1 sec. This time varies greatly with temperature. In frog muscle, at a temperature of 4 or 5°C., the contractions are greatly prolonged. With rise of temperature the contractions become shorter in duration, until a temperature of about 20°C. is reached. With further rise in temperature the duration of the

contraction is not appreciably altered until, at about 35°C., the muscle proteins begin to coagulate, when the contractions are markedly diminished in amount and duration. Above 40°C. the muscle has no excitability. The striated muscles of different animals show wide variations in the duration of the simple contraction. In some of the cold-blooded animals, the duration may be as long as one second. In mammals, the simple contraction of most of the muscles lasts less than 0.1 sec. The most rapid contractions are those occurring in the wing muscles of certain insects. These contractions may occur in as short a time as 0.003 sec., according to some estimates. The time of the simple contraction varies in different skeletal muscles of the same animal. These variations are found to conform, in general, to differences in structure of the several muscles. Muscles, having a comparatively large proportion of the so-called red fibers, for example, muscles of dark meat of birds, tend to be slower in their rate of contraction, while muscles having a comparatively large proportion of pale fibers, as in white meat of birds, tend to contract much more rapidly. Although the differing occurrence of red fibers and pale fibers is especially marked in the skeletal muscles of birds, similar differences are found among those of all vertebrates and correspond to differences in rapidity of contraction.

The Latent Period.—The electrical variation occurs, as explained above, during the latent period. It marks the spread of the wave of excitation over the muscle cells. The process of excitation results in the development of tension in the normal muscle. It is difficult, perhaps, to conceive the meaning of the term, development of tension. Bayliss has suggested an analogy that is helpful in picturing this change. He suggests that the resting muscle may be likened to a coiled lead wire, which, if stretched out retains the shape thus produced. An excited, normal muscle, on the other hand, he likens to a coiled steel wire which, if stretched, shows tension, so that it tends to spring back to its original coiled shape upon release from the stretching weight. The change that occurs in a muscle during its latent period of contraction is comparable to that which would occur if the coiled lead wire acquired the properties of a coiled steel one under tension. This tension, once developed in excited muscle, is bound to be followed by contraction unless the muscle is attached to a weight greater than it can move. The amount of tension developed in a given muscle can be measured by finding the weight just large enough to prevent the muscle from shortening after excitation. When a muscle develops tension under these circumstances, that is, without shortening, the process is called an **isometric contraction**, since the muscle fibers retain the same length.

Absence of Oxidation during Development of Tension.—Tension is not produced in the muscle as a result of any explosive, oxidative reaction although the comparatively large expenditure of energy that occurs dur-

ing muscular contraction might lead one to suppose that such was the case. Hill and others have shown that much of the heat given off by a contracting muscle is produced during stages later than the latent period, that is, after tension has developed. More than half of the heat produced by muscular contraction is evolved after shortening has begun and has no relationship to the development of tension. This part of the heat of contraction is called by Hill the "recovery heat," because it is produced by the processes which prepare the muscle fibers for another contraction. This recovery heat is not produced when the muscle is excited in an atmosphere of nitrogen, that is, in the absence of oxygen. The smaller part of the heat of muscular contraction, the part produced during the development of tension and called the "initial heat" is evolved even when the muscle contracts in the absence of oxygen, so that it appears to be the result of chemical reactions other than oxidation. The mechanical work in the muscle, then, is not the immediate result of oxidative heat production. Further proof of this fact is found in the ability of muscle to contract in the absence of oxygen. Fletcher showed that frog muscle in an atmosphere of nitrogen gave a maximal contraction every five minutes during two and one half hours before it showed any signs of fatigue. Fatigue does come on, to be sure, much sooner in a muscle deprived of oxygen than it does in one supplied with it. Moreover, a muscle once fatigued does not recover, in the absence of oxygen, although the excised muscle largely recovers from fatigue in air and a muscle provided with a good circulation of blood, entirely recovers in a short time. Fletcher also showed that muscle, contracting in an atmosphere of nitrogen, gives off no more CO_2 than could be liberated from the bicarbonates dissolved in muscle juices. Oxidation in a muscle is thus shown to be a recovery process rather than a part of the contractile process proper.

Lactic Acid Production during Development of Tension.—What is the chemical change causing the development of tension? It is probably the sudden production of lactic acid in some part of the muscle cell. A variety of lactic acid, called sarco-lactic acid, has long been known to be present in dead muscle (meat). Sarco-lactic acid has the same formula, $\text{CH}_3\cdot\text{CH}(\text{OH})\cdot\text{COOH}$ (α hydroxy propionic acid), as the acid produced by bacterial fermentation of lactose during the souring of milk; but sarco-lactic acid is dextro-rotatory, while milk-lactic acid is optically inactive because it consists of the racemic form. If excised muscle is made to contract vigorously, during the entire period while it is dying, it may contain as much as 0.4 per cent of lactic acid. A smaller proportion is found in muscles which die while the circulation is still intact, because the circulation removes the lactic acid, in part, and favors the oxidation of part of it to CO_2 and H_2O . Lactic acid is not present in detectable amounts in living, resting muscle, nor in living, active muscle under conditions suitable for oxidation. This absence of lactic acid is difficult to

prove, because the contractions, incident to dying processes, are accompanied by production of lactic acid which is not apt to be removed or oxidized under these conditions. But Fletcher and Hopkins found that if muscle is quickly removed from an animal, immediately after killing, plunged into ice cold alcohol and ground up under the alcohol, the muscle is found upon analysis to contain only the merest trace of lactic acid. This shows that, although lactic acid is fairly abundant in dead muscles, it is all produced during the dying stages. Inasmuch as the amount of lactic acid, thus produced, is noticeably increased by exhausting stimulation of excised muscles just before death and particularly increased if the stimulating and dying occur in the absence of oxygen, the conclusion is that lactic acid production in any dead muscle is incidental to death contractions (*rigor mortis*). The natural inference is that lactic acid is also produced as an accompaniment of normal contractions. Fortunately, direct proof is available. Since the time of duBois-Reymond, it has been known that some kind of acid forms in a contracting muscle. This fact is shown by the fuchsin-frog experiment. A frog is several times injected with the dye called acid-fuchsin, until the blood contains an adequate, though not lethal, concentration of this substance. After pithing the animal the muscles of one leg are made to contract vigorously by induction shocks applied to the sciatic nerve. The muscles of this leg, in contrast to those of the other (resting) leg, take on the red color that is characteristic of acid-fuchsin in acid solution. During such an experiment the hydrogen ion concentration of the muscle substance may change from pH = 7.43 (an average figure for resting muscle) to pH = 6.84. Although this change may seem small, it requires considerable quantities of lactic acid to produce it in the presence of the various buffer substances of living cells. Hopkins has further shown that the acid, thus demonstrated to be present in excited muscle, is really lactic acid. He was able to prove this by the use of his delicate thiophene test for lactic acid. There is also evidence of lactic acid production during muscular activity in the intact body. Men have been found to excrete more than the usual trace of lactic acid in the urine after extreme muscular effort, such as sprinting, during which oxidative processes were probably insufficient to dispose of all the lactic acid formed.

Lactic acid production is incidental to the development of tension and is not dependent upon the actual process of shortening because lactic acid is formed in a muscle when shortening is prevented by excessive weighting. This chemical process must be of explosive suddenness. The entire latent period for development of tension may be less than 0.01 sec.

Glycogen is a precursor of the lactic acid. This is shown by the fact that glycogen disappears from a muscle during its activity, so that any muscles that have been exhaustingly worked just before the death of the animal contain a much smaller proportion of glycogen than do other

(rested) muscles of the same animal. It is very difficult to imagine, however, any chemical reaction that would produce lactic acid from glycogen with explosive suddenness. Older ideas on the subject tried to account for the reaction on the basis of a transformation of glycogen into glucose and subsequent change of glucose to lactic acid. Both these changes are known to occur under the influence of specific enzymes found in numerous different animal tissues. But these enzymes do not cause explosively rapid reactions, so that it seemed necessary to look for some other mechanism to account for this reaction. This problem has assumed a new aspect as a result of the recent work of Embden and his co-workers. They have discovered in muscle a compound that is very unstable and is readily changed into lactic acid. They have succeeded in showing that this compound is a union of some kind of a hexose group with two phosphate groups. They therefore call it a **hexose diphosphate** and assign to it the formula $C_6H_{10}O_5 \cdot (PO_4)_2$. This substance appears to be the immediate precursor of sarco-lactic acid. Embden supposes that glycogen reacts with phosphoric acid in the muscle to produce a certain amount of hexose diphosphate, which is probably present in the resting muscle cell only in some complex combination, called "lactacidogen." This unstable compound suddenly breaks down upon excitation of the muscle to liberate lactic acid and phosphoric acid. The heat produced by this reaction together with that produced by partial neutralization of the lactic and phosphoric acids in their reactions with buffer substances of the muscle, such as bicarbonates and proteins, is probably sufficient to account for the "initial heat" of muscular contraction. Embden assumes these reactions to be reversible, so that all of the liberated phosphoric acid and that part of the lactic acid, which does not suffer oxidation, are changed back again into hexose diphosphate with the aid of glycogen stored in the muscle. Embden's theory seems all the more plausible in that acid phosphates, chiefly H_2KPO_4 , are known to be given off by a muscle worked beyond the limits that permit normal, complete recovery—that is, complete reversibility.

Irrespective of the details of the mechanism, lactic acid and probably phosphoric acid are produced in the excited muscle. Changes in hydrogen-ion concentration, thus arising, are supposed to cause a sufficient change in the behavior of the complex colloidal structure of muscle substance to bring about the development of tension with consequent tendency to change of shape in the muscle.

The Contraction Period.—When tension becomes sufficient to overcome inertia of the muscle and its attached weights, the latent period ends and the second, or shortening period begins. This change of shape signifies an alteration in the disposition of materials in the muscle. Elaborate investigations have been undertaken in order to find the exact location within the muscle cell where this alteration occurs, and also to

find what substances change their position. It is generally conceded that the dark bands of the fibrillae are increased in volume while the muscle contracts. (Fig. 75.) This can best be seen if the muscle fibers are viewed, during contraction, under a polarizing microscope. Whether the increase in volume of the dark bands is at the expense of the light bands or of the sarcoplasm is not yet certain, since different observers are not in agreement upon this point. Such minute details are exceedingly difficult

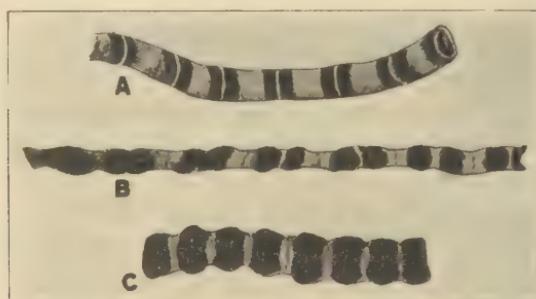


FIG. 75.—Changes of shape in striated muscle fibers. Fibrils from the wing-muscle of the wasp. *A*, relaxed. *B*, stretched. *C*, contracted. (After Schäfer.)

to make out in whole muscle fibers and observations of muscle sections necessitate the use of fixatives whose altering effects cannot be interpreted with certainty. The substance which goes into the light bands, so as to increase their volume during contraction, is assumed to be water because it is known to change its location during the swelling of proteins, which occurs when they are in contact with water or water solutions. Water is also known to move across semi-permeable membranes, such as doubtless exist in all protoplasm, and to move rapidly in response to sudden changes in osmotic pressure. A sudden localized change in hydrogen-ion concentration might readily alter the swelling powers of proteins (attraction for water) or by changing the dissociation of proteins, might alter osmotic pressure of certain definitely localized regions or it might alter both of these forces. In any case, movement of water would result. In one sense, the swelling of proteins is itself an osmotic phenomenon, in that the colloidal character of the proteins prevents their free diffusion. They therefore draw water to themselves much as do other molecules and ions when restrained from diffusion by a semipermeable membrane. These, however, are not all the forces that might possibly account for development of tension in the excited muscle. Localized changes in surface tension might conceivably enter into the process. The fact that the amount of tension is actually increased by lowering of temperature, within certain limits, thus exhibiting what is called a negative temperature coefficient, indicates that the force of surface tension is in some way concerned in the process; because surface

tension is the only force known to have a negative temperature coefficient. All these possibilities and some others have been discussed as mechanisms of muscular contraction and attempts have been made to test them experimentally, but evidence sufficient to establish any of them is still lacking. Possibly each of these theories holds some part of the truth.

External Work.—The phase of shortening of the muscle is the time when external, useful work is done. The amount of this work depends upon the size of the muscle, upon the kind of muscle, upon its nutritive condition and upon external circumstances. The relationship of size of muscle to its working capacity can be determined by finding the weight it just fails to move (absolute power of the muscle) and comparing this weight to the area of the cross-section of the muscle. The resulting figure is surprisingly large for some muscles. The relationship of the nutritive condition of the muscle is such that the larger its store of glycogen, the longer it may be kept at work without renewed supplies from the blood. Sugar of the blood is doubtless the immediate source of glycogen renewal,

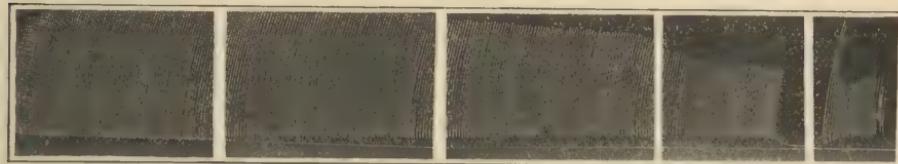


FIG. 76.—The effects of repeated contractions upon the behavior of frog muscle. The muscle was stimulated through its nerve with uniform induction shocks at 1 sec. intervals. Parts of the record, uniform with adjacent parts, are omitted. The first few contractions show progressive increase in height (the "treppe" or "staircase"). For a time the muscle then gives uniform maximal contractions. Later on the height of contraction falls off owing to fatigue. At the same time the muscle shows failure to relax completely (contracture) after each contraction. At a stage of noticeable fatigue, a period of rest is followed by a maximal contraction showing partial recovery; but the height of contraction soon falls off and contracture reappears.

although fats and, to a less extent, proteins appear to be indirectly available for muscle fuel, in that the body is capable of producing sugar from them. The rapid restorative effect of sugar for fatigued human beings has been clearly demonstrated. Another condition, which markedly affects the working power of the muscle, is its hydrogen-ion concentration. The height to which a given weight is lifted by a muscle, during a simple contraction, increases progressively in the course of the first five or ten contractions which follow a resting period. (Fig. 76.) Because of the appearance of the graphic record of such contractions, this phenomenon is called the "treppe" or staircase. Lee has shown that if a certain concentration of lactic acid is perfused through the muscle before the initial contractions are made, the very first contraction will be a maximal one and no "treppe" will occur. It appears, therefore, that there is a certain optimal hydrogen-ion concentration at which the muscle has maximal

efficiency, so that the "treppe" results from the development of acid during the initial contractions, without its complete removal after each individual contraction, up to a time when the optimal hydrogen-ion contraction is attained. When this occurs the processes of contraction may be continued with the nearly perfect maintenance of an equilibrium of hydrogen-ion concentration so that lactic acid is removed, for a time at least, at the same rate that it is produced.

The working efficiency of the muscle is also related to the amount of load which it moves or the resistance overcome. With no load upon the muscle, its contraction does no external work. With a measurable weight upon the muscle, external work is done. The amount of this work can

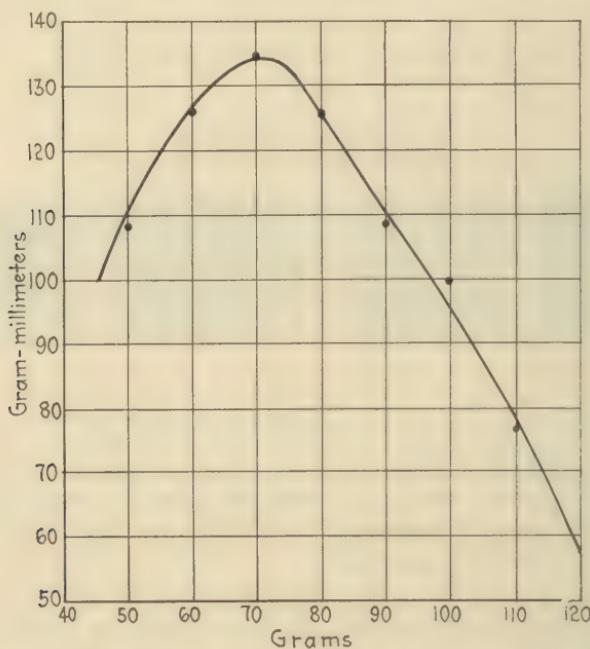


FIG. 77.—The effect of load on the work done by a muscle. Frog muscle, excited by uniform induction shocks, gave single contractions each of which raised a definite weight. The weights used in the separate trials are shown in the abscissas of the curve. The ordinates show the work done. The maximum occurred, in this experiment, with a load of about 70 grams.

be computed in the usual way by multiplying the weight by the vertical distance through which it is lifted. For example, if a frog gastrocnemius lifts 20 gm. to a height of 5 mm. it does 100 gram-millimeters of work. If the muscle is made to give a series of single contractions, with different weights attached to it in each case, the gram-millimeters of work done show a maximum at a certain optimum load. Any load, smaller or larger than this optimum, will result in the performance of a smaller amount of external work. If the work done in each of such a series of contractions

is plotted as ordinates against the respective loads as abscissas, a curve such as is shown in Fig. 77, is obtained. Muscle, like man-made machinery, does its most efficient work when carrying a load that is neither too small nor too large.

Heat Production.—Heat is given off by the muscle during the contracting phase. This heat is only a part, however, of that developed as a result of the entire contractile process. The amount of heat produced is largely determined by the conditions under which contraction occurs. If, for example, the muscle is loaded to such an extent that it cannot shorten at all, all of the energy, liberated as a result of excitation, will be converted into heat. But if the muscle is permitted to contract under optimal conditions of hydrogen-ion concentration, load, temperature, etc., the part of the energy appearing in the form of external work will be the largest possible so that heat will constitute the smallest possible proportion of the total energy. This condition, then, gives the maximum of working efficiency for the muscle.

The Relaxation Period.—The process of relaxation is not a mere pulling out of the muscle to its resting shape by external forces. It is not comparable to the fall of an unsupported weight under the action of gravity. Relaxation is a reversal of the process of contraction and as such involves activity dependent upon definite chemical or physico-chemical changes within the muscle cells. This fact is shown by a study of the relaxation time and its variations. The time required for relaxation is often longer than the contraction period. Moreover, these two periods are not affected to the same degree by certain changes in the conditions of the muscle. Cold prolongs the relaxation period of frog muscle much more than the contraction period. During the onset of fatigue in a muscle, responding to regularly repeated single stimuli, the relaxation phase is so prolonged in proportion to the contraction phase that the muscle does not completely relax between the separate stimuli when they are given, say, once per second. (Fig. 76.) This failure to relax completely is called **contracture**; if caused by cold it may be called cold contracture; if by fatigue, fatigue contracture. It may be due to the failure of complete removal of lactic acid from the muscle fibers. At any rate, accumulation of fatigue products, lactic acid, CO_2 and acid phosphate appears to bring on contracture. Probably, the awkwardness and poor control of a fatigued, human muscle is partly due to fatigue contracture. The failure of muscle relaxation that occurs in swimmer's cramp and similar spasmodic, uncontrollable contractions of muscles has not been investigated; so that the extent to which fatigue contracture may contribute to such conditions is unknown.

Another entirely different type of contracture is produced by the alkaloid, veratrin. Figure 78 is the graphic record of contraction of the gastrocnemius of a frog that had been injected with veratrin. The record

shows that the contractile phase is unaffected by the presence of the drug, while the relaxation phase is enormously prolonged. This veratrin contracture indicates that the drug acts upon the muscle so as to slow down some specific reaction or reactions that occur during the relaxation phase. The veratrin effect is antagonized by a certain concentration of fatigue products in the muscle; so that a veratrinated muscle, that is made to contract repeatedly at short intervals, soon shows a normal



FIG. 78.—the veratrin effect on muscle. Contractions of the gastrocnemius of a frog which had been injected with veratrin sulphate. The time line gives 0.5 seconds. The first contractions show marked prolongation of the period of relaxation although the muscle begins to relax as usual. Repeated contractions largely and progressively abolish this effect; but after a period of rest, it reappears.

duration of the relaxation phase. If the muscle is then rested, during some five or ten minutes, the next contraction that it gives will again show veratrin contracture. Evidently a certain hydrogen-ion concentration antagonizes the veratrin effect; while restorative processes of rest, which oxidize lactic acid and permit the resulting CO_2 to diffuse away from muscle cells, give veratrin an opportunity to produce contracture again.

A certain proportion of the total heat produced in a contracting muscle is liberated during the relaxation period. This fact indicates that oxidation of lactic acid to $\text{CO}_2 + \text{H}_2\text{O}$ is going on at this time. Restorative processes, which prepare the muscle cell for subsequent contraction, are thus shown to take place during this phase. The restorative processes involve not only oxidation but also, as suggested above, the reversal of the reaction by which lactic acid is produced. According to Embden's theory, four-fifths of the lactic acid is changed back again into a hexose diphosphate. Although the processes of oxidation, as indicated by the time relations of heat production, go on during both contraction and relaxation periods, they are not necessarily completed until a measurable time after the muscle has come to rest.

The physico-chemical processes, by which something, presumably water, changes its location in the muscle cell during contraction, must be reversed during relaxation. If it is true, for instance, that water moves from the sarcoplasm into the dark bands of muscle fibrillae during con-

traction, this water doubtless moves in the reverse direction during relaxation.

Summary of the Nature of the Contractile Process.—The following resumé presents, in brief, the nature of the contractile process in skeletal muscle as it appears in the light of our present knowledge. Excitation causes the sudden liberation of lactic acid and probably phosphoric acid (acid phosphates) from some very unstable compound in muscle substance. This compound is probably the hexose diphosphate described by Embden. This reaction produces changes of C_H^- at some definitely localized parts of the cell. This change causes the development of tension in the muscle fibrillae. Why the tension develops is very difficult to say; but apparently a change in the location of something, presumably water, occurs. The tension may be the result of an increased attraction for water by muscle colloids, chiefly proteins. It may be the result of sudden changes in osmotic pressure. It may be the result of a sudden change in surface tension. It may be true that each of these forces is, in part, responsible for the development of tension. In any case, the tension once produced causes a change of shape in the muscle by shortening of the fibrillae. Immediately after acid is produced in the muscle the processes of restoration begin. These include oxidation of part of the lactic acid and restoration of the remainder to its original state. This restoration involves the using up of some of the glycogen store of the muscle. With disappearance of acid, tension decreases and the muscle relaxes.

Rôle of Proteins and Other Muscle Constituents in Contraction.—Are materials, other than carbohydrates, phosphoric acid and lactic acid, concerned in the chemistry of muscle activity? Inasmuch as the entire physico-chemical structure of a muscle cell must be intact, if contraction is to occur, we naturally conclude that muscle proteins and many other diverse cell constituents in some way enter in to the contractile process. This does not necessarily mean, however, that they take part in such a way as to be used up or changed in an irreversible way. They may be imaginatively regarded as the muscle structural mechanism, comparable to the cylinders, pistons, valves, etc. of a gas explosion motor; for, although such structures are not destroyed or otherwise irreversibly changed, they are absolutely requisite for working activity of the motor. Just as there is a certain amount of wear and tear on the metallic constituents of a motor and, under some circumstances this break down is very considerable, so in the muscular mechanism the structural components are not entirely unaffected by activity. It still remains true, however, that carbohydrate is the chief fuel for the muscle as is gasoline for the motor.

The proof that proteins are not normally used up for purposes of energy production in active muscles is obtained from observations on the

composition of the urine. This is possible because the nitrogen-containing compounds, such as urea and ammonium salts, which result from protein catabolism, are chiefly excreted by the kidneys. The lack of special fuel value in proteins is indicated, in the first place, by the failure of the animal to lay up any reserve store of protein. This failure is recognized by comparison of total protein intake, or, what amounts to practically the same thing, the total nitrogen of the food with the total output of protein catabolism products, which is practically equal to the total nitrogen of the urine. In a healthy human adult, under normal conditions of living, the nitrogen intake per day is constantly equal to the nitrogen output. This fact is expressed by the statement that the body tends to preserve **nitrogen equilibrium**. During growth or recovery from any wasting disease the body retains a part of its nitrogen supply, thus showing what is called a plus balance. This retention represents a synthesis of new body protein but does not normally occur in healthy adults. Even a very high intake of protein food does not cause the storage of a protein reserve that would be useful were proteins required as fuel for muscular work. Further proof that proteins are not used is found in the maintenance of nitrogen equilibrium irrespective of the amount of muscular work. If a man maintains a steady level of protein feeding and his diet also contains adequate amounts of carbohydrate, his total nitrogen excretion will be only very slightly less during a day of complete rest than it is during a day of even such strenuous work as climbing a mountain. It may be the same on the two days. If the man is not in a well-nourished condition or is not in a state of muscular training, adequate for the work he undertakes, a distinctly increased nitrogen excretion will result from muscular work. During starvation, muscular work sometimes increases nitrogen excretion. Any extreme and exhausting muscular exertion is liable to cause marked increase of nitrogen excretion even in a well-nourished person.

In general, muscular work is done at the expense of foods other than proteins. Carbohydrate is used chiefly. Fat is used to some extent, but there is no clear evidence to show the extent to which fat, as such, is used in the muscle. Transformation of part of the fat into carbohydrate is possible in the animal just as is the reverse transformation of food carbohydrate into storage fat. It is quite probable, therefore, that when fat is used, during muscular work, part of it is transformed into carbohydrate before it contributes to actual energy production in muscles. Inasmuch as certain amino-acids can also be transformed so as to yield sugar, proteins can furnish energy for muscular work in this way. The possibility that muscle may utilize fat and protein, as such, has not been excluded definitely; but the marked adaptation of muscle to the combustion of carbohydrate favors the view that it is the chief and perhaps the sole direct source of muscular energy.

Compound or Tetanic Contractions.—Muscular contractions, as used for motion and locomotion, are obviously not mere twitches lasting only a fraction of a second as does a simple muscular contraction, but are sustained for indefinite periods. All muscular contractions, as they normally occur in the animal body, are sustained. They are called compound or tetanic contractions. They are possible because of the tendency of the contractions of individual muscle fibers to fuse, that is, their effects are added together. This addition is demonstrated by the process called summation of muscular contraction (Fig. 79). This process occurs when two stimuli are given in rapid succession to a muscle-nerve preparation. Effects of a second stimulus are added on to those of the first one so that

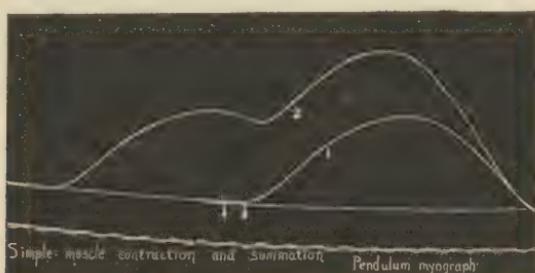


FIG. 79.—Summation of muscular contraction. Curve 1 shows a simple contraction due to a single stimulus. The latent period is indicated at the beginning of the contraction. Curve 2 shows the summation due to two succeeding stimuli. (From Howell's "Text Book of Physiology," W. B. Saunders Co.)

the muscle shortens to a greater degree and the total time of contraction and relaxation is prolonged. Evidently, some of the fibers that are not thrown into contraction by the first stimulus are effectively excited by the second one. This is at least the explanation that is in accord with the application of the "all or nothing" principle to the contraction of muscle fibers. The failure of skeletal muscle fibers to conduct excitation to one another as effectively as do cardiac muscle fibers is shown by the fact that summation occurs even though the first stimulus is a maximal one. Evidently a single artificial stimulus is not able to excite all the fibers of a given muscle.

If more than two stimuli are given to a muscle in rapid succession, a partial fusion of all the resulting contractions occurs. That is, each successive contraction takes place before relaxation is completed. The more rapidly the stimuli of such a series are repeated, the more nearly do the resulting contractions tend to fuse until, at a rate of about twenty stimuli per second, for frog muscle-nerve preparations, contraction is steadily maintained with no sign of relaxation between the separate stimuli (Fig. 80). This sustained contraction is called **tetanus**. The rate of stimulation required to produce complete tetanus varies with the kind of muscle

and with its condition. Any delay of relaxation (tendency to contraction), such as occurs when the muscle has a hydrogen-ion concentration above the optimum, diminishes the rate of stimulation necessary for tetanus.

Although the graphic record of complete tetanus shows no waverings but has a smooth plateau indicating a sustained contraction of the muscle,

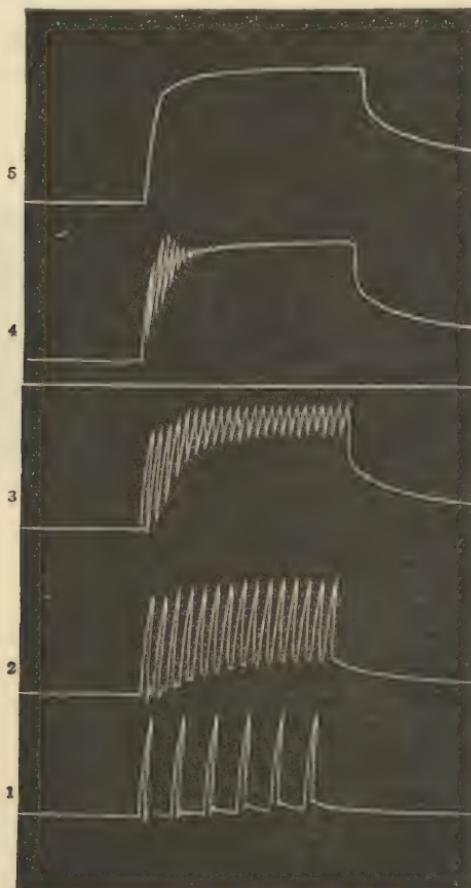


FIG. 80.—Tetanus as a result of rapidly repeated stimuli. Contractions of frog gastrocnemius with varying rates of stimulation. For record 1, the rate was sufficiently slow to permit the muscle to give separate contractions for each stimulus. With more and more rapid rates of stimulation, the contractions tend to fuse until, in record 5, a sustained contraction or tetanus is obtained. (From Howell's "Text Book of Physiology," W. B. Saunders Co.)

tetanus is nevertheless a discontinuous process. This might perhaps be inferred from the necessity of rapid repetition of stimuli to produce it. But more direct evidence is available. A musical note, the **muscle-tone**, is given off by a muscle in tetanus. It can be heard by applying the ear to a contracted muscle, though a stethoscope is usually required to magnify the sound. The pitch of this note is such as to indicate that the

muscle fibers give off vibrations at the same rate that stimuli are administered. A possible interpretation of this correspondence is that each of the successive stimuli throws only a part of the fibers of a muscle into contraction and the twitching of the different groups of fibers, thus separately excited, creates the rhythmic vibration within the muscle. A record of the electrical variation of a tetanized muscle (current of action) bears out this idea for the galvanometer string gives nearly regular

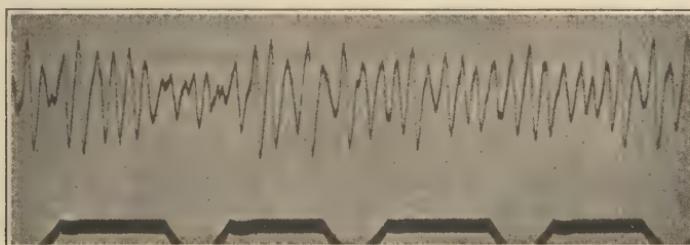


FIG. 81.—String galvanometer record of tetanus. The upper curve is a photographic record of the vibrations of the string during a voluntary contraction of the flexors of the fingers. The lower curve shows 0.2 sec. intervals. By comparison with this, the galvanometer curve is seen to record electrical variations in the muscle at the rate of 50 per second. (*From Piper.*)

oscillations during the tetanus and thus shows that excitation is occurring rhythmically in the muscle. (Fig. 81.) The reason that tetanus appears to be a sustained process, when it is really a discontinuous one, is merely that the number of fibers in contraction at any one moment is maintained very nearly uniform. The relaxation of any one group of fibers is only partial and is not effected before contraction of another set begins.

Voluntary contractions, even when very rapid, are of the tetanus type. The inference is that such contractions are produced by a rhythmic series of nerve impulses coming from the central nervous system. Use of the string galvanometer, with electrodes applied over the skin near certain muscles, shows the rhythmic activity of voluntary contractions in man. The rhythm varies from about 40 per second in certain leg muscles to about 100 per second for the jaw muscles. The rhythm is constant, within narrow limits, for any given muscle, irrespective of the force of its contraction. The variation among the different muscles indicates that the nerve centers are each characterized by a certain definite rate of discharge of motor impulses into efferent nerves; so that any center, aroused by reflex excitation or by an effort of will, always sends out a series of impulses at a definite rhythm so long as it is being excited. The rhythmic passage of these impulses along motor nerves can be shown by observation of the string galvanometer with electrodes applied to a motor nerve of an experimental animal.

The steadiness with which tetanus can be maintained in a given group of muscles varies in different individuals. A quantitative method of

testing the comparative steadiness has been devised. The apparatus used consists of a metallic plate, perforated by holes of different diameters, and a fine metallic needle. Both plate and needle are connected to wires from a battery in such a way that if the needle comes in contact with the plate, an electrical circuit is completed. A recording device, included in the battery circuit, signals each contact made. The person, whose steadiness is to be tested, holds the needle in successively smaller holes of the plate and the number of contacts that he makes, unavoidably, while trying to hold the needle in the center of the hole, is recorded. The smaller the hole, in which he can avoid more than a minimal number of contacts during a given time or the total number of contacts, that he makes in holes of a given size, furnishes a quantitative measure of the wavering of his muscles. Application of this method of testing to army candidates for training as sharp shooters has shown that those who naturally possess the greatest steadiness develop into the best marksmen with the least expenditure of time for training in rifle target practice. Those who are most unsteady, in accordance with this test, appear to be unfitted by nature for accurate shooting, since even prolonged training may not develop them into good marksmen. These observations indicate that the natural rates of discharge from the motor nerve centers under the influence of the will are much more perfectly adjusted in some individuals than in others.

The Proportion of the Energy Liberated That is Utilized for External Work. Mechanical Efficiency of the Muscle.—The total energy liberated during a muscular contraction appears in at least three different forms, heat, mechanical work, and electrical energy. The last is so small in amount that it may be disregarded in a computation of the mechanical efficiency of the muscle, which is computed, as in the case of any engine, in terms of the proportion of the total energy utilized for external work. The heat, evolved in a contracting muscle, can be measured by means of a thermo-couple and expressed in terms of calories. The work done can be measured and computed as described above. Its amount can be translated into thermal units by making use of the mechanical equivalent of heat which makes one calorie equal to 426.5 gram-meters of work. In this way it is found that the heat calories are two or three times as much as the work calories. If a machine develops three times as many heat calories as work calories, it is said to be 25 per cent efficient. From this standpoint, then, the muscle is about 25 to 33 per cent efficient. The efficiency varies, however, according to the condition of load, temperature, etc. described above. It also varies for different muscles and for the same muscles in different animals. Under the most favorable conditions, it may run as high as 40 per cent for some muscles. In general, tetanus contractions are more efficient than single ones. Zuntz claims that the muscles of the human body are adapted to work with

their best efficiency in walking and especially in lifting the weight of the body as in mountain climbing. He also claims that the efficiency of a given group of muscles is increased by training; so that if muscles are used repeatedly for the same acts, they are able to do this particular work with a minimum loss of energy in the form of heat.

The heat produced in muscles is not entirely wasted because it is the chief source of heat for maintenance of body temperature.

Phenomena of Fatigue.—The evidences of the onset of fatigue in muscle include a diminished height of contraction and a tendency to contracture (Fig. 76). There is also a progressively decreased working power of the muscle. In extreme fatigue, the muscle actually loses excitability,

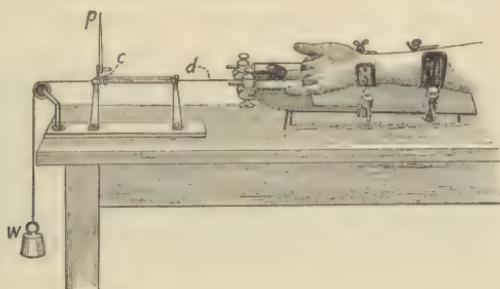


FIG. 82.—The Mosso ergograph. *W*, the weight to be lifted. *C*, attachment of cord for moving weight over the pulley. *d*, cord for attachment to the finger. *p*, point for writing on recording surface. (From Howell's "Text Book of Physiology," W. B. Saunders Co.)

although if the loss is complete, it probably represents an irreversible change and should be looked upon as death, rather than as mere fatigue. In any given stage of fatigue, a muscle may be unable to lift a certain weight which it previously moved, but it can still lift a smaller one.

The cause of fatigue is the accumulation of waste products. This is shown by the fact that a rested muscle shows fatigue abnormally soon, if it is exposed to sufficiently high concentrations of CO_2 or lactic acid and also by the fact that abundant circulation of well-oxygenated blood wards off fatigue. The latter fact is most easily shown by the use of the **ergograph** (work recorder) which registers the work done during voluntary contractions of human muscle. There are many different forms of the ergograph and of the similar type of apparatus called the dynamometer (strength measurer). The form of ergograph, devised by Mosso (Fig. 82) has been very widely used in physiology. Inasmuch as good circulation permits oxidation of lactic acid and removal of CO_2 , fatigue of a muscle in the body is thus warded off much longer than it is in an excised muscle. Also a given rest period may permit complete recovery of a muscle *in situ*, while the same rest period would allow an excised muscle to recover, only partially, from a corresponding stage of fatigue. Moreover, fatigue of excised muscle is more steadily progres-

sive, in that the heights of its successive contractions, under uniform conditions, fall off regularly; while a muscle, provided with a good circulation and not excessively loaded, comes to what is known as a "fatigue level" at which it maintains a certain height of contraction for a long period, although this height is less than that shown by the muscle when fresh. During the stage of "fatigue level" the processes of muscle restoration are adjusted to those incidental to a given amount of work performance so as to maintain a fairly constant equilibrium of conditions in the muscle. In other words, waste products are removed as rapidly as formed.

The cause of extreme fatigue, from which an excised muscle shows no signs of even partial recovery during rest, doubtless includes a complete destruction of muscle glycogen, although other muscle substances are probably also depleted. Of course, waste products are markedly accumulated in such a muscle. Similar abnormal depletions and abnormal accumulations of waste products undoubtedly occur even in muscles *in situ*. For example, the soreness that appears in overworked muscles, especially in a person not in training for the type of exercise taken, indicates some change in the composition of muscle—a change not easily reversible. An hour or two of vigorous tennis playing by a person that has been leading a sedentary life, may produce muscle soreness which lasts several days. The changes that cause this condition are not definitely known. One might suspect that the structure of the muscle cells or their nerve endings is actually altered in a slowly reversible manner. Changes, amounting to actual disruption of muscle fibers, can be produced in an excised muscle by overloading; but such disruptions are obviously not the cause of the muscle soreness which follows prolonged vigorous exercise without lifting of excessive weights.

The first effect of fatigue appears to be exerted upon the **receptive substance** near the **motor end plates** of the nerves. This is shown by experiments on the frog muscle-nerve preparation; for, after stimuli have been applied to the nerve until the muscle no longer responds by more than the merest trace of a contraction, the same strength of stimulus, directly applied to the muscle, causes a fairly vigorous contraction. This shows that the muscle is not really exhausted at the time when it appears to be no longer responsive to nerve stimuli. As explained above, the nerve fibers are likewise not fatigued under these circumstances. The inference is that some substance, which forms a physiological link between nerve fibers and muscle substance, must give way first. This would appear to be a provision of nature, tending to prevent exhausting overwork of a muscle in somewhat the same way that the blowing out of a fuse prevents the overloading of the circuit of an electrical appliance.

Fatigue in the Central Nervous System. Psychological Aspects of Fatigue.—Fatigue as we ordinarily recognize it in ourselves is often not muscular fatigue, even at times when we believe it to be such. The sense

of fatigue is very complex and involves psychological factors not subject to quantitative measurement. Fatigue is oftentimes associated with states of mind. We call these lack of interest, lack of will power, laziness, etc. The dispelling of the sense of fatigue, by music and other forms of entertainment, is a familiar psychological experience. Such results represent changes in the activity of structures in the brain that are concerned only indirectly in muscle activity. Actual physiological fatigue of the motor nerve centers in the brain and spinal cord, centers which directly influence muscular activity, undoubtedly occurs. In some cases it may even take place before any true fatigue has appeared in the muscular apparatus itself.

The varying resistance of the central nervous system to fatigue and even that of the muscular apparatus itself is largely determined by the

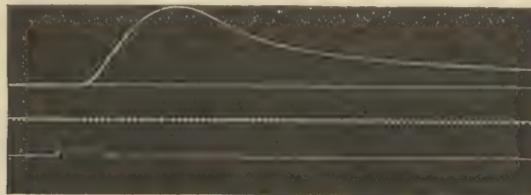


FIG. 83.—Contraction of non-striated muscle. The time line records seconds. The stimulus was given at the moment of the first break in the lowest line of the record. After a considerable latent period, a slow and prolonged contraction is given. (From Howell's "Text Book of Physiology," W. B. Saunders Co.)

behavior of secretions from the ductless glands, thyroid, adrenals, testes, ovaries and some others. The effects of these internal secretions are chiefly indirect and are exerted through their action upon the circulation and upon the general nutritive condition of the body. The physiology of the ductless glands will be discussed in a later chapter.

Contraction of Visceral Muscle.—The general importance of the activity of visceral (non-striated) muscle is appreciated when one remembers that the digestive system, the blood vessels and some other important organs are supplied with this kind of muscle. All of the so-called vegetative functions of the body are in some way dependent upon the activity of visceral muscles so that these, unlike the skeletal muscles, are indispensable to maintenance of life.

General Characteristics.—Activity of visceral muscles is characterized by slow and gentle contractions. The actual shortening is believed to occur in the fibrillae of the muscle cells. If this is true, the lack of cross-striations, such as occur in skeletal muscle, may be responsible for the slowness of their contraction as contrasted with that of skeletal muscle. All the phases of contraction of visceral muscle are more prolonged than those of skeletal muscle. The latent period, after an artificial stimulation, may be 3 seconds or more in duration and the total time of contrac-

tion, in response to a brief stimulus, may be 20 seconds or more (Fig. 83). Visceral muscle shows sub-maximal contractions in response to weak stimuli and maximal ones in response to stronger stimuli. The threshold stimulus is always large as compared with that required for skeletal muscle. Visceral muscle shows summation in response to two successive stimuli and a prolonged contraction, resembling tetanus, in response to repeated stimuli. Owing to the slowness of its activity, visceral muscle can be brought into this state of sustained contraction by a relatively slow rate of artificial stimuli. Muscles of the frog stomach, for example, are made to contract in this manner, by stimuli given once in 5 seconds. Visceral muscle, on the whole, is much more sluggish and less excitable than skeletal muscle.

Tonus.—Muscular activity, in which a shortened condition is steadily maintained for a prolonged period, is called muscle tonus. Although all muscles exhibit this type of activity, or at least something akin to it, visceral muscle is preeminent in its power to show tonus. This word is derived from "muscle tone" and hence implies rhythmic activity such as occurs in tetanus. Indeed, the word tonus is applied to any physiological process that is sustained in such a way as to suggest that it is due to regularly repeated (rhythmic) excitation. We speak therefore of tonus or tonic activity of nerve cells, of glands and of all kinds of muscles. If the conception of rhythmic excitation be strictly adhered to, then tonus is not the right word for sustained shortening of visceral muscle, but the word is so generally applied in this sense that it will be used here. We shall even call it the true tonus to distinguish it from other tonic activities which, unlike this one, are necessarily dependent upon repeated excitation. The importance of visceral tonus is obvious if one bears in mind the fact that muscles in the walls of arteries must remain in an at least partially contracted condition throughout life. It is very largely the effect of tonus in these muscles which maintains a satisfactorily high blood pressure that is one of the requisites of vigorous health. Likewise, the muscles of the stomach and intestine must maintain an at least partially contracted condition throughout all periods of healthy digestion. The popular term "good tone" as used to signify vigorous health, is almost equivalent to the physiological term, tonus of visceral muscle. The prolonged contraction of this type of activity would entail an enormous expenditure of energy were it not for the fact that the mechanism in the muscle which maintains tonus is entirely different from the mechanism of tetanus.

Tonus has been studied with conspicuous success in the muscles of bivalve molluses. The muscle which closes the shells in these animals, is wholly or partly of the non-striated kind. The power, with which this muscle holds the shells together, is appreciated by anyone who has opened oysters. But the most remarkable aspect of the activity of this muscle

is the long time that it is able to remain contracted. Moreover, the bivalve muscle can sustain heavy weights during long periods. A clam, for example, suspended by one shell, with a heavy weight hung on the other, can remain closed several days. Metabolism of the animal is not increased by thus holding up a weight. Oysters, clams, scallops or mussels use no more oxygen and produce no more carbon dioxide, while the bivalve muscle holds up a weight in this manner, than they do when the muscle is relaxed. As such animals can feed only when the shell is open, they are fasting throughout the entire period of the weight-holding experiment. Yet, computations made by Bethe, show, that if these molluscs did this kind of work by a mechanism, similar to that of tetanus in striated muscle, they would use up an amount of carbohydrate (glycogen) larger than the weight of the entire animal in order to hold up the weights hung upon them during such experimental periods. Similar computations show that the arterial muscles of a mammal, working by a tetanus mechanism, would use up more than one-fourth of all the fuel consumed in the ordinary metabolism of the body. As these muscles comprise only a very small part of the weight of the active tissues of the body, the consumption of fuel in arterial muscles must be only a small proportion of the whole. The unremitting contraction of the artery walls is due, however, to tonus and not to tetanus. Tonus, whether it occurs in visceral muscles of mammals or in similar non-striated muscles of other animals, is a very economical process. Although energy is expended during the actual shortening process, the shortened state is maintained without expenditure of energy.

The lack of oxidative metabolism as an accompaniment to tonus shows that it is really a shortened condition of the muscle due to a temporary internal change in the structural arrangements and not to an active dynamic process. In other words, the shortening, once attained, is held without effort. It may be likened to the sustaining of a weight by a ratchet device for, although one would have to do work, while actually lifting the weight, it could be maintained at a given height without effort, if the ratchet caught and supported it. With this analogy in mind, Von Uexküll called the tonus mechanism of muscle a "Sperrung" for which Bayliss has substituted the English term "catch" mechanism.

This mechanism and the contrast between it and that of tetanus has been especially investigated by Von Uexküll in the Pecten (scallop). In this animal, the bivalve muscle is a double organ. It really consists of two entirely distinct muscles lying parallel but separate. One of them, the larger, is striated. The other is non-striated. The activity of these two muscles is very different. The striated one contracts and relaxes rapidly, thus producing a rapid closing and opening of the shells. This occurs when the animal is first removed from the water and causes a flapping of the shells, which lasts for a few moments. It also occurs when

the animal is under the water and moves from place to place, if disturbed or if in search of food, for the shell opens and closes in such a way that the resulting water currents give locomotion to the animal. When the Pecten is sufficiently disturbed, as by removal from the water, its shells, after a few flappings, close tightly and, like a closed oyster, can remain shut for a long period. This sustained closure is due to the non-striated muscle. The relationship between the action of the two muscles is curiously shown by allowing the shells to close with a piece of wood between their edges. The wood is held very firmly and only by wrenching and twisting can it be pulled out. After it is removed, the shells do not further close. They remain, as Von Uexküll says, like the jaws of a vise, after a piece of wood has been wrenched from their grasp. The shells can be further closed, however, by the lightest pressure of the finger. If partially closed in this way, they take up this new position and hold it with vise-like firmness. "The muscle fibers seem to have been suddenly frozen solid," says Von Uexküll. It thus appears that the rapid contraction of the striated muscle can be followed up by a comparatively permanent shortening (tonus) of the non-striated muscle. The first works by the tetanus mechanism, the second by the "catch" mechanism. This fact is further shown by cutting one or the other of these two muscles. Cutting of the striated muscle leaves the shells still firmly closed, but cutting of the non-striated muscle causes the shells to sag open. The striated muscle can maintain closure only while it is in tetanus and this process lasts only during constant stimulation. Moreover, the tetanus is not a very powerful contraction so that it may entirely fail to lift a weight which could be held up by tonus in the non-striated muscle during a very long time.

The control of the bivalve muscle in any of these molluscs must provide for both excitation and inhibition of the non-striated muscle. This dual control is provided by their nervous systems. The actual controlling mechanism has been studied in a few Lamellibranches. Results of such studies show that stimulation of one group of nerve fibers, coming to the muscle via a certain path, causes shortening, while stimulation of another group causes relaxation. In the normal activities of the animal, both the excitatory and inhibitory nerves are reflexly stimulated. A similar dual control of viscerai muscles in mammals was described above (p. 265).

Sustained contractions are by no means peculiar to non-striated muscle; but the sustained contractions in skeletal muscles are tetanus under all ordinary circumstances and, therefore, are dependent upon the receipt by the muscle of a stream of nerve impulses. All contraction ceases in skeletal muscle after complete severing of nerve connections. Sustained contractions also cease during deep sleep, as is shown by the complete softening and sagging of the muscles of a sleeping person. Skeletal tonus

is at least diminished during light sleep or any other conditions of bodily relaxation and is to a certain degree under control of conscious acts of the will. During waking hours, a large proportion of the skeletal muscles maintain a slight degree of sustained contraction that is commonly called tonus, but at all times, this type of tonus is really a mild tetanus resulting from continuous reflex excitation or from rhythmic discharges of motor nerve cells. The only tonus of skeletal muscles, which is comparable to that of non-striated muscle, is the type occurring in fatigue contracture, when acid in the muscle is sufficient to maintain its fibers in a partially shortened condition without steadily repeated excitation.

The actual mechanism of true tonus is not understood. The analogy to a rachet or "catch" mechanism was not intended by Von Uexküll or by other investigators to suggest anything more than the fact that energy is not expended during tonus. There is, of course, no simple, mechanical contrivance of this sort in non-striated muscle. The fact that a certain degree of acidity in a skeletal muscle causes fatigue contracture, which is very much like tonus, suggests that there is, in non-striated muscle, some physico-chemical arrangement by which acid, once produced so as to cause a certain degree of shortening, can be retained in such a position as to maintain the shortened condition. The actual force—protein swelling, osmotic pressure, surface tension or whatever it may be that is set at work by a certain disposition of hydrogen ions, is probably the same in any kind of muscle. A satisfactory explanation of the tonus mechanism awaits, then, a complete understanding of the mechanism of muscular contraction in general. So far as a solution of this problem has been reached, it invites the tentative assumption that the difference between a simple contraction and a tonus is that, in the case of the latter, tension once developed is maintained because of the absence of an immediate reversal of the chemical reactions and the physico-chemical changes that produce tension.

The processes, producing tension in non-striated muscle, can be reversed, however. This follows from the fact that tonus can be made to decrease or to disappear. This relaxation occurs, for the most part, as a result of inhibiting nerve stimuli, although abnormally high concentrations of CO_2 and some other environmental conditions can also abolish tonus.

Rhythmic Contractions of Visceral Muscles.—Superimposed upon tonus of visceral muscles is another type of contractile activity: A slow contraction and relaxation repeated rhythmically at regular intervals. Several different methods have been employed to study this kind of activity. In one method, a loop of the intestine of a dog or a cat is removed from the animal, placed in warm Tyrode's solution and kept at body temperature, while a recording lever, attached by a thread to the wall of the intestine, registers muscular movements of the preparation. Rhythmic activity or "beating" of the intestine soon sets in and may

keep up, regularly, during several hours. A graphic record of such an observation is shown in Fig. 69. Similar records are obtained with a piece of the ureter and some other visceral organs. Another method for demonstration of rhythmic activity consists in taking a series of X-ray photographs of the stomach and intestine during normal digestion after a "bismuth meal." This method yields something analogous to a moving picture of digestion. The details of digestive movements will be described later. Suffice it for the present to call attention to the fact that these movements are many of them rhythmical. Another method sometimes shows rhythm in arterial muscles. This method is the observation of blood pressure. It sometimes reveals slow, rhythmic changes of pressure caused by regular contractions and relaxations of the muscles of the artery walls.

The origin of rhythmic activity is in the nervous system. The rhythm of arterial muscles, for example, can be obtained only when their controlling nerves are intact. Even the rhythms in an excised intestine are dependent upon the action of a plexus of nerves and nerve ganglia contained in the walls of the intestine. This nervous control of non-striated muscle is able to produce rhythmic activity because the nerves are both excitatory and inhibitory and may therefore cause alternating increases and decreases of tonus.

The possibility that rhythmic beats originate in non-striated muscle itself independently of the action of the nervous system cannot be excluded. If contractions did originate in this way they would be called myogenic in contrast to neurogenic. Observations of what appear to be myogenic rhythms have been made but, in every case, they occur under conditions that are so abnormal that they give no indication of the true physiological behavior of the muscle.

Cardiac Muscle.—The action of cardiac muscle resembles that of striated muscle in some respects, that of non-striated in others. The rate of contraction is intermediate between the other two types. Excitability is about equal to that of striated muscle. The facility with which cardiac muscle conducts excitation, has already been described. This property results in a remarkable demonstration of the "all or nothing" law. Cardiac muscle is incapable of tetanus because it has a long refractory period. Application of ordinary, tetanizing, artificial stimuli to the heart merely causes an increased rate of beating without tetanus, although the tonus of the heart muscle is increased by such excitation. Cardiac muscle is like non-striated muscle, in that it can be directly inhibited by stimuli that reach its cells through inhibitory nerve endings. Cardiac muscle shows a considerable degree of tonus, which is demonstrated by the fact that during vagus stimulation, the heart relaxes more than it does between normal beats. The muscles of the heart are probably in a state of slight tonus throughout life.

The particular and characteristic feature of the activity of heart muscle is *rhythmicity*. It possesses the intrinsic power to originate contractions. It is automatic, self-excitatory, to a degree not observed in any other kind of muscle. This is not true of the hearts of all animals for in some of the invertebrates, at least, heart beats are dependent upon rhythmic stimulation by the nervous system. In such animals, however, histological examination shows the heart muscle to be unlike typical, cardiac tissue but to resemble skeletal muscle. The origin of the heart beat will be discussed further in Chap. XIX.

Amoeboid Motion.—The movements of an amoeba were described above in connection with protoplasmic streaming. This type of movement occurs in all the so-called naked protozoa, in the cytoplasmic masses inside of plant cells, in leucocytes of all animals and in embryonic nerve cells.

Attempts to discover the ultimate nature of the process of amoeboid movement have been undertaken. Investigations have been especially directed to an effort to correlate it to changes in surface tension. Such changes can produce movements of drops of mercury that are not unlike amoeboid movements. Anything which diminishes surface tension on one side of the drop, will permit the still undiminished tension on other sides to force the mercury to move. The applicability of this and similar observations to the explanation of amoeboid motion is, however, very doubtful. It is not really known to what extent surface tension enters into this process any more than its rôle in muscular contraction is known. Certainly the conditions on and near the surface of protoplasm are much more complex than they are upon the surface of a drop of mercury. Not only surface tension but other properties of protoplasm, for example, viscosity, are subject to marked and sudden changes. Changes in viscosity, as explained above, are attributable in part, at least, to changes in the behavior of proteins and therefore would result from localized changes in hydrogen ion concentration, resulting from excitation. Probably changes in the disposition of water, such as appear to occur in contracting muscle cells, are also produced in the outer cytoplasmic layers of amoeboid cells (p. 246). If this is the case, the chief difference between amoeboid motion and muscular contraction lies in the fact that the latter effects only certain definite and restricted changes in shape because the movements of water occur only in relation to highly specialized structures, the muscle fibrillae.

Ciliary Motion.—The waving or vibratory motion of the microscopic hairs or cilia, that occur on certain types of living cells, is called ciliary motion. These slender processes are of two main types: (1) Cilia proper, which are comparatively short and usually occur in large numbers upon each cell which bears them and (2) flagella, which are much longer than cilia and occur in small tufts or as single hair-like processes.

Cilia are found on protozoa and on the ciliated, epithelial cells of all animals (Fig. 84). Ciliated epithelium occurs, in general, in the lining of certain hollow organs or passages that is, upon mucous and similar membranes. In some animals, especially invertebrates, ciliated cells are found in the lining of various portions of the digestive system. In the higher animals, ciliated cells are found in the lining of the respiratory passages: Bronchial tubes, trachea, pharynx and nasal passages; in the lining of some of the genital and urinary passages: Fallopian tubes,

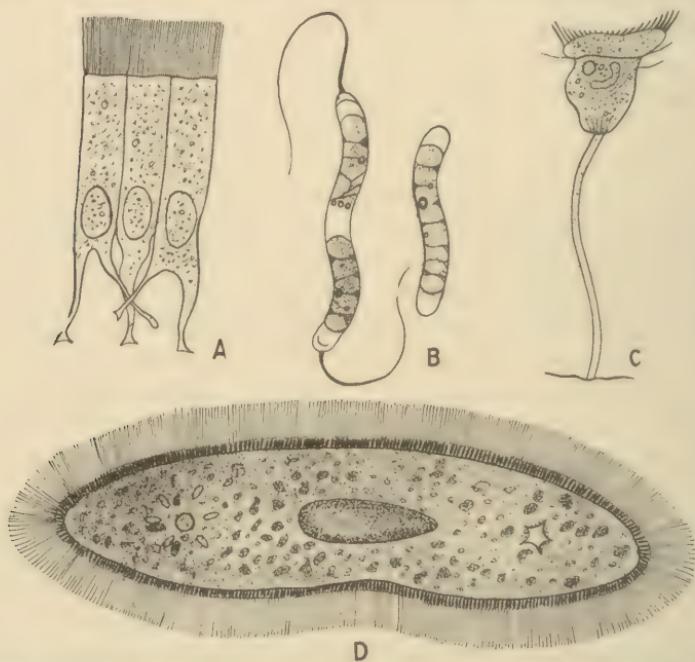


FIG. 84.—Types of ciliated cells. *A*, ciliated epithelium from the frog. *B*, flagellated protozoan. *C*, vorticella. *D*, paramoecium.

uterus and tubules of the epididymis; in the lining of the tear ducts; in the lining of the Eustachian tube and portions of the middle ear; in the lining of the ventricles of the brain and the central canal of the spinal cord.

Flagella are found on certain kinds of bacteria and other microorganisms and also form the tails of spermatozoa of all animals.

The motions of cilia can be conveniently observed in a portion of an oyster gill. This organ is extensively provided with ciliated epithelium, so that a small piece of gill tissue, taken from a recently opened oyster, is seen under the microscope to have many ciliated surfaces. The cilia move so rapidly that one can see only a hazy blur where they are located; but, as the tissue is dying or when it is cooled, the cilia move more slowly so that one is able to make out waves of motion, passing over them in

much the same way that waves of bending motion sweep over a wind-blown grass field (Fig. 85). When the ciliary motion has slowed down sufficiently in the dying tissue, one can make out the character of the bending process in each individual cilium and can see that it bends suddenly in one direction, thus taking on a hooked shape, and then slowly unbends. The force of its movement is exerted almost entirely in one direction, that of its quick bending.

The object attained by ciliary motion upon epithelial surfaces is the motion of liquids on solid particles that are in contact with them. In respiratory passages, mucous secretions, dust, etc. are moved towards the exterior, because the quick bending of the cilia is always in a direction producing this result. Respiratory passages are thus kept cleared. Upon the gills of oysters and similar invertebrates, the cilia create a constant current in the sea water surrounding the gills and also move food particles along the surfaces of the gills toward the mouth. In the pharynx and oesophagus of the frog and numerous other animals, cilia move food particles toward the stomach. In the Fallopian tubes and uterus, ciliary motion is supposed to direct the movements of spermatozoa toward the ovum.

Cilia are able to produce such purposeful results because their activity is perfectly coordinated. No one cilium bends independently of the action of the others but always in relation to its neighbors; so that the waves of activity progress along the ciliated surface in the same direction as that of the quick bending of the separate cilia. This progression is due to the conduction of excitation from cell to cell in the ciliated surface. This conduction is very efficient for it has been shown to occur in groups of ciliated cells even after their cilia have lost motility. The coordination of ciliary action is not dependent upon external regulation such as nerve impulses. Indeed, the cilia attached to a small portion of cytoplasm, severed from the cell by micro-dissection, will continue to act in perfect synchrony during a considerable time after being thus cut off.

The amount of work accomplished by the cilia is surprisingly large. It can be measured conveniently by the use of a preparation of the ciliated surfaces from the mouth and oesophagus of the frog. After dissecting the mucous membrane from the roof of the mouth, it is removed with the oesophagus still attached to it. The oesophagus is then split lengthwise along its ventral side and the entire ciliated surface, thus obtained, is stretched out and pinned upon a cork surface. The cilia maintain activity for some time if kept moist with isotonic salt solution. Small objects, such as pieces of cork, move along this surface toward the stomach end of the oesophagus. Metallic weights, if laid upon cork chips that rest upon the cilia, will be moved in the same direction, even though the prepara-



FIG. 85.—Diagrammatic representation of the waves of bending motion in cilia. (After Verworn.)

tion is placed so that the weights have to be moved up hill. Weights as large as 500 milligrams can sometimes be moved by fresh preparations. By measuring the vertical distance through which these weights are lifted, the work done by the cilia, per unit of time, can be computed in terms of milligram-millimeters. Such computations show that, as in the case of muscular work, there is a certain optimum load, with which the cilia do maximal work. Smaller or larger loads give less efficient results. The rate, at which the cilia move, is greatly increased by rise in temperature within physiological limits. If, for example, the ciliated surface is irrigated with a physiological salt solution at 28°C., objects resting upon the cilia will move much more rapidly than they would at room temperature. Temperatures slightly above 30°C. destroy the cilia of the frog. The rate of ciliary action is also markedly affected by changes in hydrogen-ion concentration of the surrounding medium. A C_H⁻, slightly on the alkaline side of neutral, is optimal for their activity; while a slight acidity, though at first hastening their motion, soon slows them down. The amount of work that the cilia can do is largely determined, then, by the loading, the temperature and the hydrogen-ion concentration. In these respects ciliary action resembles muscular contraction.

The motion of flagella can be seen best by microscopic observation of spermatozoa. Flagella, like cilia, move so rapidly under physiological conditions that they cannot be seen; but when in a dying state or when cooled, they can be seen to move in an undulatory way, sometimes described as a "whip-lash motion." This propels the flagellated cell through surrounding liquids.

The work of flagella and of the cilia of protozoa when done for the purpose of locomotion of the organism is large in proportion to the minuteness of these appendages. The work of the cilia of the Infusorian, *Paramoecium*, has been calculated from the movements of individuals. This work is sufficient to raise a weight about nine times as great as that of its own body.

The nature of ciliary motion is fundamentally like that of muscular contraction. The cytoplasm, on one side of an individual cilium or flagellum contracts, thus giving the change of shape in the form of a sudden bending. Probably, the mechanism producing this result consists of a sudden change in the location of water as in other forms of protoplasmic movement. A slow reversal of the contraction processes causes a gradual unbending of the cilium or flagellum. Although muscular contraction, ameoboid motion and ciliary motion have been described separately here and appear very different to casual observation, yet the fundamental activity in each case is a change of shape of some part of the protoplasm of a cell. The most satisfactory view of the nature of the process is that which attributes these activities to localized alterations in the disposition of water. According to this view, all protoplasmic movement is essen-

tially of the same character and the apparent differences in behavior are due to differences in the detailed structure of the cell.

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CHAPTER XIII

THE FUNCTIONAL UNITS OF NERVOUS SYSTEMS: NEURONES AND PROTONEURONES

Although the physico-chemical structure of protoplasm renders it able to respond to environmental changes, the responses of the protoplasm in many types of cells are not called forth by any great diversity of changes or by minute ones. This is due in part to the nature of cell surfaces. Naked protoplasm, exposing a living surface, as in the amoeba, has more opportunity to respond to stimuli than has protoplasm which is surrounded by protecting walls, such as the cellulose structures of plant cells and the sarcolemma of muscle cells. Failure to respond is due, at least in many cases, to the lack, within the cell, of a structure sensitized to respond to certain environmental forces. An ordinary muscle cell, for example, is not responsive to visible light, because it contains no photo-sensitive substance capable of responding to the rays of the visible spectrum. The great responsiveness of living things and especially of the highly organized animals is possible only because of a nervous system. There are two fundamental advantages afforded by a nervous system: (1) Nerve structures are so constituted physico-chemically that they are especially excitable, that is, they respond to exceedingly minute forces, (2) nerve structures are peculiarly well constituted to favor conduction of excitation. This power enables them to transfer an excitation from cells that are especially sensitized to a given stimulus into cells which respond by motion and other activities.

Effectors and Receptors.—Organs or other cellular structures, which respond to excitation by some definite activity that is useful to the organism, are grouped together under the term effectors. Although muscles and glands are the chief effectors, light producing organs, such as those of the fire-fly, and electrical organs, such as those of the torpedo ray, should also be classed as effectors. Effectors are, in general, less excitable than are nerve structures.

Organs, so constituted that they are especially sensitive to certain definite stimuli, are called receptors. They constitute the end organs, receiving the stimuli which arouse sensory nerves. The obvious examples of receptors are the special sense organs, those of touch, taste, hearing, sight, etc. But less familiar examples are met with in physiology: Photo-sensitive spots of invertebrates, chemical receptors of fishes, pressure receptors of certain plants and some others.

One of the functions of the nervous system is to serve as the connecting link between receptors and effectors. As found in its simplest form, in some of the invertebrates, the nervous system appears to have no function other than that of conducting and distributing excitation.

Neurones, Sensory and Motor.—Nerve fibers do not exist independent of nerve cells but are always formed, during embryological development of animals, as outgrowths of the cells. Moreover, any nerve fiber severed from its connection with its cell body, dies and suffers complete structural degeneration. For these reasons, a nerve fiber is not regarded as a functionally independent structure but the nerve cell with its out-

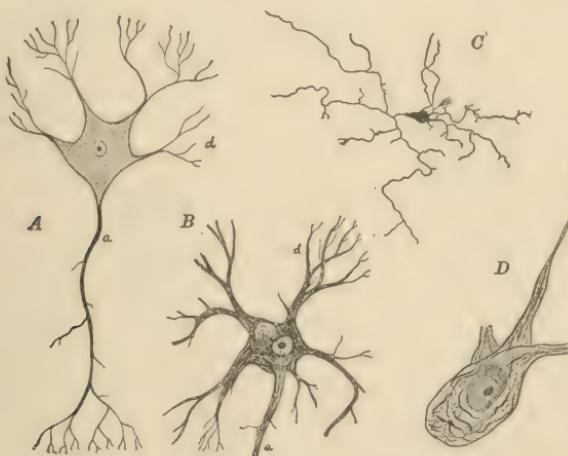


FIG. 86.—Types of neurones. *A*, diagram of a typical neurone. *a*, axis cylinder process or axon with terminal arborizations. *d*, dendrites. (After Obersteiner and Hill.) *B*, cell from human spinal cord. (After Obersteiner and Hill.) *C*, nerve cell from the eye. (After Lenhossek.) *D*, nerve cell from the earthworm. (After Kowalski, from Sharp's *Cytology*.)

growing fibers is looked upon as the physiological unit of the nervous system. The majority of the actual cell bodies are located in gray matter of the nervous system and their attached fibers run through the white matter and the trunks of nerves. Certain nerves of larger animals connect the central nervous system with distant organs. Fibers in such nerves may be three feet or more in length; but even in these extreme cases, each fiber is an outgrowth of its nerve cell body. The entire structure, consisting of one nerve cell and a varying number of attached fibers, is called a neurone. Some typical neurones are represented in Fig. 86. The different fibers branching off from the nerve cell behave in specific ways in that they normally conduct impulses in only one direction. This is the basis of the statement that nerve cells possess *polarity* which simply means that impulses enter nerve cells only by way of certain of their processes and leave only by way of certain other processes. This distinction is well exemplified in the type of nerve cell shown at *a* in

Fig. 86. It possesses numerous short processes, which receive impulses. As all of these are branched, they are called **dendrites** (tree-form). Only one fiber conveys outgoing impulses in this type of neurone. This is the long **axon fiber**, also called a **neurite**, connecting with an effector. Such a neurone, directly connected with an effector and therefore having direct control over processes of contraction and secretion is called an **efferent neurone**. Neurones connected to receptors and therefore receiving stimuli directly from the sense organs, are called **sensory neurones**. Neurones are in some way provided with a mechanism which has been likened to a valve in that it permits the conduction of excitation in only

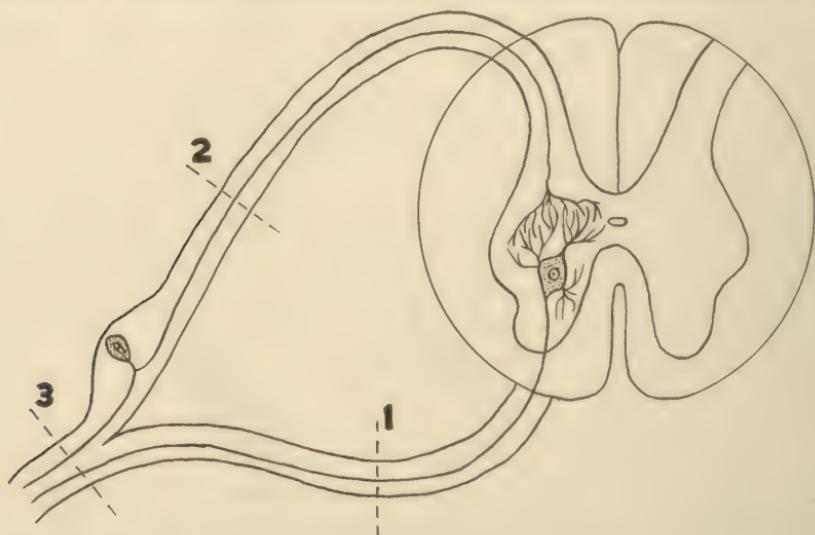


FIG. 87.—Diagram of a root of a spinal nerve. One sensory and one motor neurone are shown on a highly magnified scale. Cutting the ventral root at 1 causes degeneration of its fibers on the side of the cut distal to the spinal cord. Cutting the dorsal root at 2 causes degeneration of its fibers nearer to the spinal cord. Cutting the spinal nerve at 3 causes degeneration of all its fibers on the side of the cut distal to the spinal cord.

one direction. The chief evidence in support of this idea is the observation that stimulation of central stumps of severed motor nerves does not result in any detectable response either in the central nervous system or in other parts of the body. A further discussion of the polarity of neurones will be taken up in another connection, but any descriptive definition of neurones should emphasize their especially significant property of polarity. A conventional definition of the different terminal branches of neurone fibers conveniently indicates the direction of polarity. Dendrites are defined as the branchings which conduct impulses toward the cell body of the neurone while neurites (axon fibers) are defined as the branchings which conduct impulses away from the cell body.

Separation of Neurones in the Roots of Spinal Nerves.—Distinctions between the physiological behavior of motor and sensory neurones can

be made out more clearly in experiments on the vertebrates than on other animals, because of the separation of these two types of neurones that occurs in the roots of the spinal nerves. Each spinal nerve is connected with the spinal cord by two roots which pass through separate spaces between adjacent vertebrae. The posterior or dorsal one of these two roots (Fig. 87) has a distinct swelling or ganglion upon it. This ganglion contains gray matter which is found, histologically, to be composed largely of cell bodies. The other, the anterior or ventral root, contains only nerve fibers. It is entirely comparable structurally to any nerve trunk. The anatomist, Sir Charles Bell, first showed (1811) that the two roots have different functional connections in that stimulation of the anterior roots produces contraction while stimulation of the posterior roots is not apt to cause this effect. The French physiologist, Magendie, made the chief discoveries by which the different physiological behavior of these two roots was established. He showed (1822) that if the posterior root of a spinal nerve of a dog was severed, sensation in the skin regions supplied by that particular nerve appeared to be entirely lost. At least, the sense of pressure and that of pain were absent. The muscles supplied by that nerve, still appeared to function, however, without any true paralysis. Artificial stimulation of the central stump of the severed root produced evidences of sensory excitation and reflex muscular contractions, but stimulation of the other (peripheral) stump produced no visible results. Magendie found that if he cut the other (ventral) root of a spinal nerve complete paralysis of muscles supplied by that nerve resulted, although no evidences of loss of sensibility could be found. Artificial stimulation of the central stump of this root produced no visible results but stimulation of the peripheral stump caused muscular contractions. These two types of experiments proved, then, that sensory fibers probably occur exclusively in the dorsal roots, while motor nerve fibers are the only ones in the ventral roots. Histological evidence serves to substantiate these physiological experiments. The fibers of the dorsal root are found to be outgrowths of the cells in the dorsal root ganglion, while fibers in the ventral root are all outgrowths of cells in the gray matter of the spinal cord. The degeneration which follows cutting of spinal nerve roots bears out the physiological and histological findings. Cutting of the dorsal root, as shown at 2 in Fig. 87, results in degeneration of the fibers in the severed portion of the root that is still attached to the spinal cord. Cutting of the ventral root, as shown at 1, results in degeneration of fibers in the severed portion still connected with the spinal nerve. Cutting of the spinal nerve itself results in degeneration of all its fibers that are thus severed from their cells of origin. These various lines of evidence give conclusive proof that the fibers of the sensory and motor neurones are connected to their respective cell bodies according to the scheme represented in Fig. 87.

The functional connection between a sensory and a motor neurone might occur, theoretically, at a point in the spinal cord where terminal arborizations (fine branches) of a sensory fiber interlaced with dendrite branches of the motor neurone. Interlacing connections are recognizable by histological observation. They are called **synapses**. Their structure and behavior will be discussed in the next chapter. Actual anatomical continuity of fibers does not appear to occur at synapses. Until it is proved that continuity actually occurs, nerve impulses must be supposed to be transferred by mere contact across the interlacings of terminal and dendritic processes at synapses.

The Reflex Arc.—The nerve impulses concerned in a reflex act originate in receptors, travel through sensory neurones, go out from the central nervous system by way of motor neurones and so reach an effector. At least one sensory and one motor neurone must take part in a true reflex. The nerve structures concerned in a reflex act constitute what is called the reflex arc. The simplest possible reflex arc would be that represented diagrammatically in Fig. 87.

Connecting or Internuncial Neurones.—The simple reflex arc shown above is not really representative. There is, indeed, much doubt as to whether one sensory and one motor neurone alone ever constitute a functional reflex connection in the vertebrates. For the most part, sensory neurones connect with others which are not motor but which serve as connecting links between sensory and motor neurones or between sensory neurones and the so-called higher or conscious centers in the brain. These intermediate nerve units are called connecting or internuncial neurones. They are of diverse forms (Fig. 86) which vary in regard to the number of branched fibers attached to them. According to the number of such processes and to the intricacy of their branching, the different connecting neurones link up a varying number of sensory and motor neurones to each other or to the higher brain centers. A stimulus, entering the central nervous system through one or through a small number of sensory neurones, may be distributed to a comparatively large number of motor neurones and so cause activity in many muscles or other effectors. Inasmuch as the nerve impulse travels along nerve fibers with maximum effectiveness, if it is transmitted at all (the "all or nothing" principle) it is not uselessly dissipated by even the most widespread distribution among many neurones. Thus a single nerve impulse may not only arouse numerous motor responses but may also produce a reaction in consciousness. For example, a pain stimulation in the skin may result in vasoconstriction of numerous arteries while it simultaneously produces a sensation of pain. The vasoconstriction need not necessarily be the result of the consciousness of pain, but may be brought about by excitation of vasomotor neurones which, perhaps, receive impulses through collateral branches of the same connecting

neurones that transfer the impulses from sensory neurone to conscious centers. A schematic representation of internuncial neurones, showing their distributing connections in the spinal cord is shown in Fig. 88.

The General Plan of the Vertebrate Nervous System.—Although the activities of neurones have been most clearly demonstrated by observations on the spinal cord and the roots of spinal nerves, the arrangement of sensory, connecting and motor neurones, as found there, is believed to be representative of the general type of arrangement existing in all parts of the vertebrate central nervous system. Just as the spinal nerves contain the fibers which connect with the spinal cord, so the twelve pairs of cranial nerves contain the fibers that connect directly with the brain. But, while an apparently complete sorting of sensory and motor neurone fibers occurs in the spinal nerve roots, no correspondingly distinct separation is found at the points where cranial nerves emerge from the brain. There is, however, another type of sorting in this system of nerves. The auditory nerve is composed exclusively of fibers of sensory neurones, while the oculo-motor nerve is largely if not exclusively provided with fibers of motor neurones. Most of the cranial nerves, however, contain both kinds of fibers so intermingled that it is impossible to separate them by dissection. But from physiological evidence, the general plan of the entire central nervous system is shown to be similar to that of the spinal neurone arrangement. We may at least say that all the neurones of the central nervous system are roughly classified as sensory, motor, and connecting.

This brief account of the types of neurones and their relationships to each other in the vertebrate nervous system will serve as an introduction to the general subject of nerve physiology. The following sections will deal with excitation and its transmission in those simply organized invertebrates which possess no nerve structures and with the activities of the nervous systems of other invertebrates in which primitive neurones or neurone-like structures occur. By comparison between such activities and those of the complex vertebrate nervous system a general view of the probable evolution of the nervous system is obtained.

Independent Effectors.—Effectors that are normally excited directly without intervention of neurones or receptors show the most primitive type of excitation. Although they are much more prevalent in protozoa

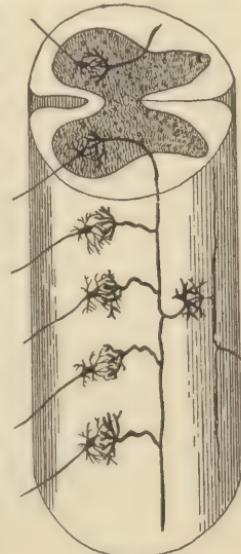


FIG. 88.—Diagram of a reflex arc to show connecting neurones. A posterior root fiber is shown entering the spinal cord at the right. Motor neurones are shown at the left. The connecting neurone is represented as distributing impulses from one sensory neurone to many motor neurones. (After Kölle.)

and the simply organized multicellular animals than in vertebrates, some examples of independent effectors are found in all types of animals. The vertebrate heart maintains rhythmic beating even though entirely severed from the nervous system. This fact would not prove its normal, independent excitability because nerve structures that are known to exist within the heart tissue might be the seat of excitation which was transmitted to the heart muscle cells. The heart of the chick embryo and of certain other vertebrate embryos begins to beat, however, at an early stage of development when no nerve structures can be found in it and those, which later on grow into the heart, have not yet reached it. Muscle cells, taken from the nerveless, embryonic heart, can be grown on artificial cultures of blood serum and in this isolated condition they continue to beat for long periods. Heart muscle cells of the vertebrates, then, originate rhythmic activity within themselves, irrespective of neurones or receptors. Cilia, occurring in all types of animals but taking a prominent part in the life of invertebrates, are another example of independent effectors. The ring-shaped muscles of the iris of the eye, muscles which on contracting narrow the pupil, are also independent effectors. They contract in response to the stimulus of bright light falling upon them directly. This is an exceptional instance of a muscle that is photo-sensitive. These particular muscle cells possess this property because they contain a pigment which is itself photo-sensitive, that is, it is chemically changed under the influence of light. The action of this iris muscle is proved to be independent of neurones because the pupil will constrict when hit by a ray of bright light even when the iris is entirely separated from all other structures except those that support it in the front of the eyeball. With numerous different types of animals it has been found that, after the front of the eye has been cut off the iris retains its power to respond to direct light stimulation.

There are, then, instances of normal direct excitation even in the so-called higher animals. But for the most part, these are exceptional cases, for the vast majority of all the cells of such animals are excited normally through the medium of a receptor-neurone system.

Contractile Structures of Simple Organisms.—In marked contrast to the rule which prevails in vertebrates, all the effectors of some of the so-called lower animals operate exclusively in response to direct excitation. This is the case at least in sponges which possess no structures that in any way resemble neurones and none that have any of the characteristics of receptors. These animals are sessile and very sluggish, but they do give certain definite responses to some types of stimuli. Their excitation has been particularly investigated by Parker. He has observed the manner in which they control the passage of currents of water through their bodies. Under normal circumstances, they maintain a flow of a large volume of water which enters through numerous minute pores scattered

over the entire surface of the animal and which passes out through a larger opening called the osculum. Movements of flagella or cilia on the linings of the cavities of the animal are the cause of this circulation of water through its body. This ciliary motion appears to be maintained constantly throughout the life of the animal, so that the only means for checking the circulation of water must be a closure of the pores or of the osculum or of both. This closing and the subsequent opening is accomplished at the pores by a change of shape of the cells that form the edges of the pores. By a sort of amoeboid motion these cells spread out to form a membrane over the pore and thus shut off the inflow of water. Cells of a different type form the lining of the oscula. Although these are not structurally like muscle cells but resemble epithelium, their change of shape is so nearly like muscular contraction that they are regarded as primitive muscular structures. When contracted, they close the oscula and therefore stop the outflow of water. The stimuli that cause a closure of either pores or oscula are mechanical injuries, inflicted near the opening, and also the presence of certain foreign substances in the surrounding water, such substances as ether or chloroform. In addition, the oscula are made to close by lowered osmotic pressure of the sea water (addition of fresh water), by rise of temperature (to 35°C.) and particularly by stagnation of the water surrounding the sponge. When currents in the water of an aquarium containing sponges are stopped, the oscula close within a few minutes.

Although this contractile power is observed around the pores and the osculum more readily than in other parts of the sponge, all the flesh cells, that is, all the soft parts of the animal are capable of contraction. Because of this power, the entire body of the sponge shrinks and wrinkles up when it is taken out of water. A change of shape thus occurs in these simple and undifferentiated body cells which is comparable to muscular contraction.

Conduction of excitation occurs in sponges as is proved by the fact that injury, such as a pin prick, to a part of the sponge about 1 cm. from the osculum causes closure of this opening without any apparent activity in the immediate vicinity of the injury. Inasmuch as no nerve structures are to be found in the sponge, such a conduction of excitation must occur by way of the non-specialized "flesh" cells of the animal. This conduction is not very effective, however. Injuries at a distance of more than a centimeter from the osculum do not result in its closure.

The physiology of excitation of sponges gives a picture of activity of independent effectors operating without any control by neurones and without any mechanism especially adapted for conducting excitation or any that is especially adapted to serve as receptors.

Simple Effector-receptor Systems.—The sluggish action of sponges is in distinct contrast to the comparatively lively responses of higher ani-

mals. Even invertebrates that are as lowly organized as hydroids, sea-anemones and jellyfishes make quick movements in response to slight stimulation. This is partly because their effectors include well differentiated muscles adapted to rapid changes of cell shape and is partly due to the possession of specialized cells that serve as receptors. In some cases, as in the tentacles of sea-anemones and certain other actinians, a simple effector-receptor combination occurs without any intervening neurone. This relationship is shown diagrammatically in Fig. 89.



FIG. 89.—A simple receptor-effector system. *r*, receptor, connecting through fibers with *m*, a muscle cell. (From Parker's "Elementary Nervous System," J. B. Lippincott Co.)

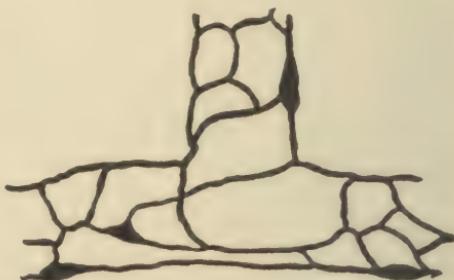


FIG. 90.—Diagram of a nerve net. The thickened places on the net represent cell bodies of protoneurones. (From Parker's "Elementary Nervous System," J. B. Lippincott Co.)

The receptor cell is located in the epithelium of the ectodermal layer and is provided with a fibrous process which has numerous branches connecting with muscles in the deeper layers of the animal. The receptors themselves are modified epithelium and are so differentiated that they are excited by contact with solid bodies or by the presence of certain dissolved substances, such as meat juices, in the surrounding water. The response to their excitation consists in movements of the tentacles to carry food towards the mouth. The many branches of the fibers of the receptor cells form a network in the layers beneath the epithelium. This network so transmits and distributes excitation that a widespread response of muscle results from even a restricted, localized stimulus.

The Nerve Net.—A more typical case of excitation in sea-anemones and one which prevails in hydroids, jellyfishes and other coelenterates, involves receptor cells and muscles or other effectors, but also includes neurone-like structures, which receive impulses from the receptors and transmit them to effectors. This mechanism is represented diagrammatically in Fig. 90. Parker has called these structures **protoneurones**. Receptor fibers have functional connections with other fibers that are outgrowths of the neurone-like cells. Many of these cells are present in

the subepithelial layers of the coelenterate body and, as each one is provided with numerous many branched fibers, the latter form an intricate network which extends through the subsurface of the animal and even penetrates into the internal structures. In some species this nerve net is present just beneath the entire surface of the animal. In others it is more restricted in its extent. In all of them it penetrates some of the internal organs.

Diffuse Nervous Transmission.—The responses of coelenterates to stimuli are, in general, of a comparatively non-specific type. In the sea-anemone, for example, Parker has shown that stimulation of the surface of the oral disk, that is, the region around the mouth, or of the surfaces of the tentacles, or of the outside of the column or main body of the animal is followed by approximately the same response. This consists of a retraction of the oral disk owing chiefly to a contraction of a group of muscles that extend lengthwise of the animal. This behavior appears at first sight like a reflex response, but Parker has shown that it differs very essentially from a reflex act. In the first place, the nervous transmission does not necessarily have to go through any one region of the animal as does a reflex act in a vertebrate. In the latter, the reflex impulse must travel over certain definite sensory neurones and be passed on, at the central exchange region in the spinal cord or brain, into certain equally definite motor neurones. On the contrary, the sea-anemone responds in a nearly normal manner to stimulation of parts mentioned above even when the entire head or the whole foot portion of the animal has been previously cut off. The same responses are also obtained when the animal has been nearly severed lengthwise either from the head downwards or from the foot upwards, provided a bridge of tissue is left intact at one end so as to preserve continuity of its nerve net structures. Excitation of these animals was tried, in Parker's experiments, after numerous other types of procedures. These included the cutting off of various parts or the making of various incisions. Results showed that, in contrast with the vertebrates, no one specific region of the animal has to be kept intact to serve as an exchange center for the nervous conduction of excitation.

A further distinction between excitation in sea-anemones and true reflex excitation is found in the fact that the former is not dependent upon any one specific sensory path or any specific connecting neurone paths. This is shown by Parker's experiments in which he cut the surface of the sea-anemone so as to produce a long, slender tongue of the dermal tissues, which remained attached to the body of the animal only by one end of the tongue. Stimulation of any portion of this tongue, even its extreme free end, produces the usual contractile response in the main body of the animal. Moreover, results are not affected by the location of the cuts that produce the nearly severed tongue of tissue nor by the direction of

the cuts, nor by the location of the tissue that serves as connection between the tongue and the rest of the body. In brief, observations show that every part of the sensory surface of the sea-anemone is in good physiological connection with every other part of the animal; so that any stimulated region can serve as the source of an impulse which can travel in any direction through the sub-dermal layers of the animal and through certain internal regions and eventually reach the same effectors, no matter where the stimulus is given. This type of conduction of excitation must occur by way of nerve structures because there are no others in this animal which have an anatomical continuity capable of providing such transmission. The many branches of the neurone-like structures of the sea-anemone constitute a complete nerve net. Transmission of impulses through it is of a diffuse character; so that, under some circumstances at least, an impulse may start from any receptor and travel, either by comparatively direct or by very circuitous paths, until it reaches muscles in the main body of the animal. The only requisite for this diffused transmission is the existence of some connecting bridge to afford a physiological continuity of the net between receptor and effector. Diffuse transmission is thus distinctly different from reflex transmission which must pass through certain definite regions in a central nervous system and must pass over equally definite afferent paths on the sensory side and efferent paths on the motor side of the central nervous system.

The Properties of the Nerve Net. *Power to Limit Conduction.*—Diffuseness of conduction of excitation through the nerve net is not without some limitation. Parker has shown, for example, that sea-anemones can give a response to stimulation by light without involving contractions of large groups of muscles. Sea-anemones respond to general illumination, such as diffuse daylight or direct sunshine, by a partial contraction of the main group of longitudinal muscles. This, like all muscular contractions in these animals, can be maintained for a long time because it is a tonus and not a tetanus. In darkness, however, the animal becomes completely expanded, because of a general decrease in its muscle tonus. But, although the response to a general illumination is a slight but uniform contraction of all the large group of longitudinal muscles, the response of an expanded animal to illumination by a ray of light directed against one side of its body is not generalized and uniform. After the latter stimulus, the muscles contract only on the illuminated side; so that the animal bends over toward the source of light, as a flower bends toward the sun. Although so specific a response does not appear to be obtainable by means of the most carefully localized pressure stimuli, this response to unilateral illumination indicates that the nerve net is not absolutely devoid of power to limit conduction of excitation. In other words, a diffuse and complete transmission over the entire net does not necessarily occur. In this respect, the nerve net suggests in a primitive

way, the much more marked definiteness of the connecting nerve paths that exist in higher animals.

Regulation of Rhythmic Activity.—Some of the other properties of the nerve net have been discovered by means of experiments on jellyfishes which possess a well developed structure of this type. The arrangement of the nerve net varies somewhat in different species of jellyfishes but, in several of them, the typical arrangement is that of a ring shaped band of nerve network, which encircles the discoid or bell-shaped animal near its margin. The nerve net is partly composed of the arborizations of fibers of the receptor cells of the animal and partly of the many branched fibers of neurone-like structures which are very numerous in jellyfishes.

Jellyfishes show characteristic rhythmic or pulsating movements due to the regular contractions of a large, ring-shaped muscle or sphincter, with fibers extending in a circular direction and forming a complete ring around the disk or bell which composes the body of the animal. These pulsations force water out of the concavity of the bell and so propel the animal through the water. The pulsations appear to depend upon excitation of the sphincter by impulses that originate in the receptor cells at the surface of the animal. This fact is proved by experiments in which the receptor cells are excised. This operation can be done conveniently upon the common species of jellyfish, *Aurelia*, because its receptor cells are all located in eight symmetrically placed spots or organs upon the margin of the bell. When these eight bodies are all cut out, the pulsations cease, at least for a time. But if one of them is left and the other seven excised, the sphincter pulsations are not stopped but occur in response to every natural or artificial excitation of the remaining receptor body. Each pulsation starts in the part of the sphincter nearest to the receptor and then continues as two waves of contraction which proceed in opposite directions around the ring muscle until they meet and die out on the side of the animal opposite the starting point.

Conduction of the excitation for this wave has been shown definitely to be accomplished by the nerve net rather than by the sphincter muscle. One proof of this is found in experiments in which the muscle ring is cut in various ways so as to impair the continuity of its fibers. This can be accomplished easily, because the muscle fibers all extend in parallel directions. Such incisions need not interfere with the functional continuity of the nerve net, because its fibers extend in every possible direction and thus preserve functional connection in spite of numerous cuttings, provided no one of them extends across the entire net. The series of incisions, as made by Romanes in one experiment of this type, is shown in Fig. 91. After such incisions, the wave of pulsation is propagated around the muscle ring in response to stimulation of the receptor; so that transmission appears to be just as effective as though the incisions had not been made. A more decisive experiment described by Bethe, can

be made with the jellyfish, *Rhizostoma*. In this animal, the arrangement of muscle and nerve net is such that incisions can be so made that they entirely sever the continuity of the muscle structure but leave a functionally connecting path through the nerve net. After this operation, the animal is still capable of responding to a stimulus with the usual propagation of the excitation. Muscle, on the side of the incision distal to the receptor, contracts in due order after the contraction of that near the receptor. Still more convincing evidence is found in regeneration experiments as made by Mayer upon the jellyfish, *Cassiopea*. After a deep wound in this animal, regeneration occurs; but the nerve is restored before those of the muscle. During a certain stage of regeneration,

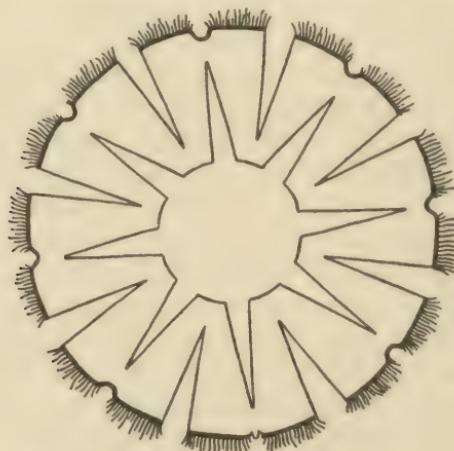


FIG. 91.—Diagram to illustrate Romanes experiment on diffuse nervous transmission.
(From Parker's "Elementary Nervous System," J. B. Lippincott Co.)

then, this animal possesses functional continuity of its nerve net over the wounded region, but has no muscles there. Under these circumstances, excitation is conducted across the regenerating portion in a perfectly normal manner. All these experiments thus agree in showing that the excitation for jellyfish pulsation travels by way of the nerve net.

Relative Conducting Power.—In another kind of animal, the Hydroid, *Corymorphia*, which also possesses an extensive nerve net and apparently no other nervous system, Parker has shown that transmission of excitation fails when the nerve net is functionally interrupted. Such an experiment can be made on this animal because its nerve net lies near the surface. When a portion of the stalk of the hydroid is locally anaesthetized it fails to transmit excitation as it ordinarily would. Muscular contractions excited at one end of the stalk of the anaesthetized animal progress only as far as the block produced by the anaesthesia, although they would

ordinarily be propagated over the entire animal. The anaesthetics used were of a kind and in a concentration known to be without effect on muscle. These experiments thus give conclusive proof that the nerve net is the transmitting and controlling structure and furthermore show that the muscle of this form is relatively deficient in its power to conduct excitation.

The rate of conduction of excitation in the nerve net is slow compared with the velocity of the nerve impulse in a vertebrate. Parker measured the rate of conduction in the sea-anemone. For this purpose he used a tongue of dermal tissue severed from the animal except at one end, as described above. By stimulating first near the free end of this strip and then near its attached end, muscular contractions are produced, but in the first case the time elapsing between stimulus and response, is greater than in the second case. The difference between these two time intervals is due to the greater distance through which the first excitation must be conducted. The difference between the two time intervals and the distance between the two points of stimulation thus give the necessary data for computing the velocity of transmission. The results obtained at 21°C. varied between 121 mm. and 146 mm. per second.

"All or Nothing Behavior."—Another property of the nerve net is its ability to transmit excitation without any diminution of its effectiveness. This is doubtless another case of the "all or nothing" principle. In the jellyfish, the ring-shaped plan of the main nerve net results in the curious phenomenon of recurrent excitation when a wave of activity, started under suitable experimental conditions, causes a pulsating contraction to course round and round the sphincter. Each portion of the muscle is able to recover excitability after contracting and so is able to respond when the excitation again reaches it after travelling around the ring. Harvey reports an observation of this sort in which the wave of pulsation travelled around the ring of *Cassiopea* during eleven days without any apparent diminution in rate. Computing on the basis of the average rate of progress of such a wave of activity it must have travelled nearly 460 miles during the time of observation.

Independent Excitability.—The nerve net, though commonly excited by impulses that originate in receptors, is nevertheless independently excitable. This is shown by experiments on jellyfish from which all receptors have been excised. Such animals can be excited so as to produce pulsating movements although the usual environmental changes are much less effective in producing this activity than they are in the case of intact animals. An especially effective artificial stimulus for the nerve net is stretching. Even a slight stretching serves to excite. Observations on the direction of propagation of the wave of excitation in nerve nets led Von Uexküll to formulate the principle that stretching so

sensitizes the net that excitation always tends to be propagated in a direction toward a more tense portion of the net rather than toward an unstretched part. This principle gives an at least provisional explanation of the apparent directive control of the conduction of excitation as often seen in nerve nets.

Quantitatively, this independent excitability of the nerve net appears to be less than that of receptors, but greater than that of muscles. The slight sensitiveness of the net, as compared with receptor excitability, is indicated by the fact that pulsations of jellyfishes cease, temporarily at least, when all receptors are excised. The probability that the net is more excitable than muscle is shown by various observations. The facility for conduction of excitation is known to be parallel, in most tissues, to the excitability. Conduction of excitation, as explained above, is distinctly better in the nerve net than in the muscles which it controls. Observations on anaesthetized animals, in which the nerve structures are temporarily inexcitable while the muscles are still responsive, gives direct proof of the advantage of a net as compared with effectors that are entirely independent of nerve. This graded series of sensitiveness: Effectors, least sensitive, nerve net, slightly more sensitive and receptors, especially sensitive, suggests, as pointed out by Parker, a picture of evolutional development in which animals having effectors alone are seen as the most primitive; while those having effectors and receptors which are connected with each other by the comparatively simple device of a nerve net, appear higher up in the scale of evolution and distinctly more responsive to their environment than are animals devoid of any nervous system.

Autonomous Action.—The independent excitability of the nerve net, together with the diffuse character of its transmission, and the absence of any well-defined, centralized connections, confers a higher degree of autonomy upon the separate parts of animals which have this type of nervous system. The tentacles of sea-anemones or of hydroids, when completely severed from the rest of the animal, exhibit the same movements that they show under normal conditions. They respond to the same stimuli, either artificial ones or those of the usual environmental kind. The presence of food causes them to make the bending movements characteristic of their ordinary feeding. In the presence of the anaesthetics, which affect nerve structures, the isolated tentacles cease motion just as they do in the anaesthetized, intact animal. This indicates that their autonomous movements are dependent upon functional activity of the nerve structures. Even small portions of the tentacles, when completely isolated, show movements typical of the behavior of the same part when present in the intact animal. The foot portion, or pedal disk, of a sea-anemone continues to creep about after it is severed from the rest of the body and such movements of the beheaded animal do not differ from the

corresponding ones of the whole animal. Numerous other instances of autonomous activity of isolated parts of animals, having a nerve net, have been recorded; so that the general conclusion is, that this kind of nervous system makes activity of parts of the body largely independent of the behavior of the organism as a whole. This is in marked contrast to the state of affairs in animals that possess a central nervous system. An isolated limb of such an animal does not respond to stimuli other than those applied directly to the trunks of its motor nerves or to its muscles. All coordinated muscular activity of the isolated part is lost. It shows no response whatever to stimulation of its receptors, such as the endings of pressure or pain nerves. Thus, autonomy of all parts of coelenterates and of other invertebrates with a similar nervous organization, is a striking feature of their nerve physiology.

Such autonomy does appear, however, in certain parts of the vertebrates. The spontaneous beating of isolated loops of the small intestine (p. 321) is comparable to the autonomous movements just described. Similar contractions occur in isolated portions of arteries and other organs containing non-striated muscle. But in every such instance, the organs possessing these autonomous powers are provided with an intrinsic nerve structure which has all the general anatomical and physiological characteristics of the nerve net of primitive animals. It appears, therefore, that while the diffuse type of nervous control, with a consequent autonomy of separate organs, is characteristic of all the activities of many invertebrates, this type of nervous control has persisted during the course of the evolution of vertebrates to only a slight degree; for in them, it occurs merely in the so-called vegetative organs, while most of the vertebrate organs are controlled by the centralized, reflex type of nervous organization.

Protoneurones.—Although the nerve net structure has been recognized in representatives of all the phyla of animals from coelenterates to vertebrates, its rôle in the life of the lower multicellular animals is much more prominent than it is in that of higher forms. In the coelenterates, indeed, it is probably the only type of nervous organization. As this occurrence of the nerve net suggests that it is the primitive type of nervous system, which phylogenetically preceded a central nervous system, Parker has designated the unit structures composing the nerve net as protoneurones. This term is useful because it suggests a resemblance between these structures and the neurones of a central nervous system and yet implies that they are more primitive than the latter. There are, indeed, marked differences between protoneurones and neurones. As explained above, the neurone has a cell body and various branches, but none of the latter have been shown to effect definite structural continuity with branches of other neurones. The protoneurone is not a correspondingly distinct anatomical unit; for although it, like a

neurone, has a cell body and numerous branches, the latter appear to be organically connected with other cell bodies of the nerve net. One thinks of a neurone as a cell and attached fibers, but a protoneurone is best pictured as a small part of a fibrous net, bearing a cell body upon each part. The diffuse character of transmission through a nerve net gives physiological evidence to bear out this anatomical interpretation of the protoneurone. Embryological evidence is also available: A study of the developmental processes of lower invertebrates shows that, throughout their embryonic stages, the cells that eventually give rise to the nerve net are constantly in close proximity to one another, so that they may possibly be in organic connection, even as they are developing.

Neurones, on the other hand, do not show diffuse transmission. One neurone may be excited without any influence upon some of its nearest neighbors, although its excitation is transferred to other neurones located at some distance from it in another part of the brain or spinal cord. The anatomical basis of this distant effect is difficult to trace; but so far as it has been made out, it is entirely in agreement with the scheme of neurone connections as outlined above and suggests that branches of different neurones grow out, not to unite with each other, but to form interlacing contacts of thin terminal arborizations and dendritic processes. The contacts, thus made, are not haphazard, but constitute definite and special reflex paths. Embryological evidence supports this idea. In many cases, the neurones which compose a definite reflex arc, as identified by physiological experiments, are known to arise during embryonic development from widely separated parent cells. The contrast between the morphology of neurones and that of protoneurones is thus a marked one, but is not as striking as is the difference between their respective physiological activities. As already emphasized, the protoneurone transmits in every direction, the neurone in only one. The latter shows polarity, while the protoneurone does not.

Protoneurone Reactions, Suggesting Polarity.—This apparent lack of polarity in the protoneurone is not absolutely complete. Some limitation of the direction of transmission has been observed in certain nerve nets. Parker has studied this phenomenon in the tentacles of sea-anemones. In one set of experiments he showed that a touch stimulus, at the tip of the tentacle, causes contraction of muscle fibers extending the length of the tentacle, but a similar stimulation of a point at the middle of a tentacle causes contraction of only certain muscle fibers between this point and the base of the tentacle. This and similar observations have led to the conclusion that conduction of excitation tends to proceed down the tentacles of these animals toward the base but is not so apt to proceed in the opposite direction toward the tip. Another type of experiment leads to similar observations. Sea water, containing a small amount of HCl, is discharged upon the surface of a tentacle with the

result that the animal is excited in such a manner that its main group of longitudinal muscles contracts and thus draws in the oral disk. But when food, in the form of fish meat, is placed on the same parts of the tentacles, they transfer it to the lips and at the same time the oesophagus is opened by the contraction of a group of muscles so arranged, radially, that their shortening distends the oesophagus. This particular feeding reaction fails not only when a wrong chemical stimulus, such as HCl, is applied to the tentacles but even when food touches parts of the body other than the tentacles. Indeed, no kind of stimulus can cause this particular response unless the receptors of the tentacles are excited. The sea-anemone, in this instance, gives a certain definite response to a particular stimulus in much the same way that a vertebrate does while performing a reflex act. The above described light response of the sea-anemone is a similar instance. Thus, a part of the nerve net of a sea-anemone can conduct certain kinds of excitation along definite nerve tracts instead of diffusing them over the whole net and, moreover, conveys impulses in only one direction as though certain protoneurones had polarity.

A possible explanation of this tendency of some protoneurones to show a limited polarity is suggested by histological observations upon the tentacles of sea-anemones and other actinians. Such observations show that the fibers, branching off from the receptor cells and conveying excitation into the nerve net, are largely and, in some species, almost exclusively arranged in such manner that they connect the receptor cells with parts of the nerve net toward the base of the tentacle. Impulses, originating in receptors which are thus connected, naturally tend to travel toward the base of the tentacle. This is the direction of their transmission indicated by physiological observations. Because of this arrangement of fibers, the muscular contractions which follow excitation of sensory cells probably begin in a part of the tentacle nearer to the base than the point of stimulation. Contractions, once started, would slightly stretch the nerve net and thus, in accordance with the Von Uexküll principle, direct the conduction of further excitation toward the base of the tentacle.

Other histological observations suggest an explanation of the instances in which the nerve net has some tendency to delimit the conduction of excitation instead of widely diffusing it. These observations show that, in parts of primitive systems, special aggregation of nerve structures form definite and partially isolated tracts in the nerve net. Such structures would tend to conduct excitation in a limited rather than a diffuse manner. Even this partially specialized nerve structure is still much less specific in its power to insure definiteness of nerve responses than is the typical neurone arrangement of a centralized nerve system. This contrast becomes very evident when the nervous reactions of coelenterates are compared with typical reflex acts such as are described in the next chapter.

The Fundamental Differences between the Primitive and the Vertebrate Nervous System.—The primitive nervous system, in which protoneurones are the units, is not centralized while the vertebrate nervous system and others, that are similarly constituted with neurones as the functional units, are centralized in the brain and spinal cord or in ganglia. For a crude analogy, one may liken the primitive nerve net structure to an interlacing system of telephone wires, so connected that a message started upon any one of them is able to pass through practically all the wires. The higher nervous systems are correspondingly likened to a modern telephone system in which central stations permit certain definite connections to be made and usually prevent crossing of wires and other conditions that might cause diffused transmission. The group of centers in which these connections are made in nervous systems is sometimes called an adjuster mechanism. The analogy between it and a telephone switch board should not be carried too far. It should not be permitted to suggest that the nerve impulse is propagated in a manner analogous to the flow of an electric current (see Chap. X), nor should the analogy suggest that the "switching" by which excitation is made to follow different paths in response to different stimuli, is accomplished by a mechanism comparable to an automatic telephone switch board which makes different connections in response to different call signals. The analogy does hold, however, in so far as it emphasizes the ability of the centralized nervous system to afford definite and special paths of conduction.

One other difference between primitive and vertebrate nervous systems is also fundamental. Protoneurones have little if any ability to limit the direction in which excitation is propagated through the net structures of which they are component parts. Neurones transmit excitation in only one direction so that they definitely limit the propagation of excitation through the central nervous system of which they are the functional units.

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CHAPTER XIV

REFLEXES

The Varying Complexity of Reflex Acts.—The theoretically simplest reflex act would be one involving a single sensory and one motor neurone. Such an act would be instigated by excitation of the sensory end of one nerve in a receptor organ and would involve activity in one effector or in a part of one controlled by one motor neurone; but such an isolated event probably does not occur. More than two neurones appear to be involved in even the simplest acts. An approach to this degree of simplicity is found in invertebrates in which certain of the reflexes involve only sensory and motor neurones. But connecting neurones in addition to these take part in the typical reflexes of vertebrates. Some exceptions to this general rule are supposed to occur. Of these, the knee-kick has received extensive study in man and laboratory animals largely because abnormalities of this response or its failure can be used as a diagnostic sign of defects, disease or wounds in certain parts of the spinal cord. The knee-kick or knee-jerk is the well-known involuntary response to a light blow on the patella ligament just below the knee. The foot kicks out because of the twitch of parts of the muscle in the front of the thigh. The muscle must be under slight tension to give a good result and this condition is attained by crossing the legs or by letting the leg hang freely over the edge of a table. A study of the latent time of this reaction, that is, the interval between stimulation and response, has shown that this reflex requires distinctly less time than do other comparable reflexes. In such comparative measurements, due allowance has to be made for the time required to excite the receptors, for the time involved in conduction of the nerve impulse to and from the spinal cord and for the latent period of muscular contraction. Subtracting these three time intervals from the total latent period of the reflex gives a remainder called the *reduced reflex time*. It represents the interval required to transfer excitation through the spinal cord from sensory to motor neurones. This reduced reflex time for the knee-kick is not more than one half that required for the typical reflexes. The shortness of this time, about 0.021 sec. as compared with 0.045 sec. for similar acts, is taken to show that connecting neurones do not function in the knee-kick while they do take part in typical reflexes.

Varying degrees of complexity occur in response to different sensory stimuli or the same stimulus under different conditions. Complexity,

as meant here, does not refer to the mere quantitative difference in the number of receptors and effectors involved, but refers rather to the complexity of the conducting path of excitation in the central nervous system. Broadly speaking, it refers to the different numbers and the different types of connecting neurones which are involved in different reflexes. The diagram shown in Fig. 88 might be greatly elaborated to show many more types of connections by way of different internuncial neurones in the central nervous system. Numerous different parts of the brain or the spinal cord can be involved in any one reflex. This is shown by the following example. Tickling the bottom of the foot produces different results under different circumstances. During deep sleep, it may produce no response. During lighter sleep, it may cause a slight withdrawal of the foot. In a person, who is awake, tickling produces conscious sensations. In this case the response consists of more extended muscular contractions which may even be convulsive or may lead to hysterical conditions. These different responses show that distinctly varying numbers of muscles are involved under different circumstances. This means that varying numbers of motor neurones are stimulated. Connecting neurones are thus shown to give entirely different distribution of excitation under slightly varying conditions and the distribution may or may not involve consciousness. Moreover, the possibilities are further greatly increased by the power of inhibition. Any one or more of numerous sensory stimuli, occurring at the same time as the tickling may entirely inhibit reflex responses to the latter. If the subject knows that the stimulating of the sole of the foot is done for experimental or diagnostic purposes, his interest in the observations may so dominate over all processes in the central nervous system, that no reflex takes place. Indeed, an effort of the will can easily inhibit the response in the majority of individuals. Inhibition shows that the propagation of excitation through the central nervous system can be diverted or blocked in various ways and by diverse conditions.

Definition of Reflexes.—Inasmuch as the response to a given stimulus may be much more complex under some conditions than under others, a definition of reflexes, distinguishing them sharply from non-reflex activities of the central nervous system cannot be made satisfactorily. The presence or absence of consciousness is not a satisfactory criterion; for, as every one knows, the same response to a given stimulus may be conscious or unconscious. For example, winking, in response to drying of the conjunctiva, may certainly be accompanied by consciousness though, in the majority of instances, it is an entirely unconscious and involuntary act. The fact that memory is involved in certain acts does not serve as an entirely satisfactory criterion to distinguish such acts from reflexes because a given response to a given stimulus may require memory at one time but not at another, at least not a conscious memory. Numerous

illustrations of such complex, unconscious activities are seen in our so-called absent-minded acts and in the performances of sleep walkers. On the other hand, some responses that are very closely akin to typical reflexes cannot occur except they involve obviously conscious sensations. Excitation of the secretion of saliva and gastric juice by the sight or the recollection of tasty food is an example of such reactions. They necessarily involve conscious memory and so are called psychic reflexes. There is a wide difference between a purely unconscious, involuntary reflex, such as those which affect the constriction of arterial muscles, and the strictly conscious, voluntary acts, such as those done in response to the dictates of acquired, aesthetic tastes. The typical reflex can be predicted; these other acts cannot. All gradations between these two extremes are to be found. But, in general, the physiological definition of a reflex lays stress upon its predictable certainty. The more mechanical and certain the act the more typical of reflexes it is said to be.

The Final Common Path.—Distinctly different sensory stimuli may evoke the same response. The sight, the sound or the touch of an approaching object or even the spoken word of warning may cause the same muscles to contract. The remarkable manner in which blind or deaf persons are able to make their unimpaired senses function in place of the lost ones is familiar. Indeed, any given set of motor neurones are excitable by various stimuli which originate in distinctly different sensory neurones. This is in accord with the above described reaction in which the same sensory stimulus affects a varying number of motor neurones because it travels over different connecting paths. But in any such case one set of motor neurones is used irrespective of how many others may be simultaneously excited. The idea that impulses, originating in different receptors, or pursuing different paths through the central nervous system, can eventually excite the same effectors is expressed by Sherrington in the phrase, "use of the final common path." Just as one or more of a large number of telephone transmitters may be connected to any one receiver, so large numbers of different sensory neurones, the transmitters of excitation, can be put in functional connection with any one motor neurone and moreover, these different connections can be made through distinctly different collateral lines of central stations.

The Reflex Frog.—After all the lobes of the brain of a frog are destroyed by pithing, without injury to the spinal cord, or when the animal is decapitated, it still responds to all stimuli provided their effects can be carried out by the use of reflex arcs in the cord. If destruction of the brain is complete, no reflexes, necessarily involving arcs that are wholly or partly in the brain, are obtainable. Such a brainless animal is called the spinal frog or the reflex frog. Cats, dogs and some other animals have been used for this and similar experiments. But the frog is especially convenient because, after a brief period of recovery from the shock

of the operation, it is very responsive to numerous different kinds of stimuli and can be used for prolonged experiments without receiving any especial care. Moreover, the pithing operation can be performed without much loss of blood so that the animal is left in good nutritive condition and the complete cessation of breathing movements, which are dependent upon integrity of the respiratory center in the brain, does not introduce complications in experiments with frogs, because their small oxygen requirements are almost completely met by extensive cutaneous respiration. The experimental object in the preparation of a reflex frog is to eliminate a large number of collateral, connecting paths through the neurones of the brain and all other connections of the brain with the spinal cord. Spinal reflexes are thus reduced to a degree of comparative simplicity. Experimental results show that this object is attained, for the animal now behaves in a remarkably mechanical way. If no environmental changes, contacts, currents of air, chemical stimuli, etc., act upon the parts of the animal supplied by spinal nerves, it remains perfectly quiescent as though it were dead. The skeletal muscles are all entirely relaxed. Muscular movements in response to any given stimulus can be predicted with an accuracy unattainable for experiments on intact animals. A light pinch of one toe causes a slight withdrawal of the same foot; a stronger pinch causes a distinct kick; violent or injurious pinching causes movements of both legs similar to those of jumping. A small piece of paper wet with dilute, acetic acid and placed on the skin of either leg or of the body calls forth remarkably coordinated movements of the muscles of either or both legs, movements so perfectly effective in removing the paper and in scratching the acid-stimulated spot that they resemble purposeful and conscious movements of a normal animal or a human being.

Characteristics of Spinal Reflexes.—The responses of the reflex frog to different stimuli are of three general types: (1) Simple coordinated movements involving contraction of a relatively small number of muscles. Although these contractions are tetanic, they are not long sustained and do not involve elaborate timing of the activity of different muscles. Examples are the brief contractions which lift the foot in response to a light pinch of the toe and the single kicking movements in response to various tactile sensations in the foot; (2) complex coordinated movements, involving the contractions of large numbers of muscles which act in due sequence to produce definite purposeful motions of a complicated character. Examples of this type are the motions for removal of acid-soaked papers as described above. Swimming motions, often produced by putting the reflex frog into water, are also complex coordinated reflexes; (3) uncoordinated or convulsive reflexes, involving violent tetanic contractions of numerous muscles which act oftentimes in opposition to one another because their contractions are not effectively timed. Flexor

and extensor muscles contract simultaneously and thus produce a spasm-like stiffening of the limbs. Such convulsive reflexes occur when very large numbers of receptors are stimulated, as is the case when the reflex animal is dipped into an acid bath. The response may be at first a coordinated, swimming or jumping movement such as would normally take place in an attempt of the animal to escape from the bath; but these movements soon change into the uncoordinated and convulsive type unless the acidified water is quickly washed off from the skin. These different types of responses show that the numbers of the excited motor neurones vary widely under different circumstances and that the manner of their excitation varies, in that the different neurones may either be excited at such time intervals that coordinated muscular contractions result or may be excited simultaneously so that convulsive and useless movements are produced.

Inasmuch as coordination of muscular movement is dependent upon an exact timing of the excitation of motor neurones and this process depends, in turn, upon the manner of conduction of excitation through internuncial and sensory neurones, the entire process of coordination is seen to be dependent upon the excitation of sense receptors. Indeed, coordinated movements are not obtained when the nerve fibers, rather than the skin receptors of a reflex frog are excited. Stimuli applied to the sciatic nerve, for example, never cause coordinated or useful movements. Even when the fibers of sensory nerves are electrically stimulated just beneath the loosened skin of the reflex frog, the resulting movements do not appear to resemble the neat and accurate ones described above. The explanation of this difference is, probably, that normal excitation of receptors arouses only certain definite sensory neurones and these determine which motor neurones are put into action and also the exact timing or synchrony of their excitation. It is therefore hardly likely that the stimulation of motor nerve fibers would produce the same result and even the stimulation of sensory fibers would hardly be expected to imitate closely the excitation of definite groups of sensory neurones that are affected by a natural stimulus.

Many of the facts relating to spinal reflexes have been shown to apply equally well to certain reflexes which employ arcs in the brain; but the latter reflexes are most of them so modified by the past experience of the animal and by the inherent factors, which are grouped together under the term instincts, that a study of these reflexes is much more difficult than is that of spinal reflexes. The winking reflex and the reflex secretion of saliva and gastric juice in response to the taste of food are among the few brain reflexes which appear to have the same characteristics as spinal reflexes. Others which may be studied by the methods used in the investigation of spinal reflexes, will be described under conditioned reflexes.

The Theory of Synapses.—Neurones have never been shown to be actually joined to each other by any anatomical connections. The interlacing networks of terminal arborizations and dendritic processes have been extensively studied by various histological methods. Although all investigators do not unreservedly agree to the idea that continuity is absent, the general consensus of opinion is that these interlacing junctions provide contacts only. Such contacts between the fibers of different neurones are synapses. Inasmuch as the unified action of the central nervous system is dependent upon the transmission of impulses across the synapses, so as to integrate the activities of different neurones, any nervous system containing neurones is called a **synaptic system**. The manner in which excitation is conducted across synapses can only be conjectured. The thin films, which are supposed to occur upon the surfaces of axon fibers, may also be postulated for the tiny branches which interlace to form a synapse. Although these films, according to the theory, do not have structural continuity from the fibers of one neurone to those of another, there must be a physiological continuity. Excitation is certainly transferred across synapses. The synapse permits excitation to be propagated across it in one direction but not in the reverse direction. This is shown by the fact, explained above, that neurones possess polarity; that is, their fibers convey impulses in only one direction. This is not a property of nerve fibers themselves, and therefore the polarity of neurones must be due either to the synapse or to the nerve cell body. Numerous physiological observations tend to eliminate the latter. Indeed, the general tendency of protoplasm to transmit excitation points to the extreme improbability that the bodies of nerve cells are responsible for polarity. Synapses, then, appear to control the direction of the propagation of excitation through chains of neurones. Synapses also determine what neurones shall be in functional connection with each other, because a given synapse is at one time open to the conduction of impulses and at another time blocks them off. The evidence for this changing behavior of synapses is given below. They are to the nervous system what switches are to a telephone station; although this comparison should not suggest that the behavior of a synapse is due to any mechanism resembling the varying conductivity of a switch for an electric current.

Reflex Time as Related to Synapses.—Although reduced reflex time has been measured in the case of numerous different reflexes, the largest number of measurements and what are probably the most accurate ones have been made on the winking reflex. The reduced reflex time for this act varies between 0.031 sec. and 0.042 sec. according to different experiments. These figures are based, for the most part, upon measurements made upon human beings. Reflex time, in the spinal animal, is determined most accurately when the electrical variation of the reflexly stimulated muscle is used to indicate the time when the muscle begins to

respond. With such a method, measurements show that when a stimulus is applied to the same leg, in which muscular responses are observed, reflex time is distinctly less than when the stimulus is applied to the other leg. This means that the crossing over of a stimulus from one side of the spinal cord to the other, consumes a distinctly measurable time, because the length of all the nerve paths exterior to the spinal cord is just the same in the crossed reflex as it is in the unilateral one. Reduced reflex time for the crossed reflex averages about twice as much as that for the unilateral one. This fact strongly suggests that considerable delay in the transmission of excitation occurs at the synapses. All reflexes which, because of their effects on consciousness, or because of other effects, appear to involve comparatively long paths in the central nervous system, show comparatively long reflex time. Although this delay is commonly attributed to the properties of the synapse, possible effects of the cell bodies of neurones in slowing up conduction of excitation cannot be eliminated as yet. Even if all of this delay were due to properties of the synapse, this would not prove that the actual "jump" of excitation from terminal arborizations to dendritic processes, consumed an especially long time, as compared with transmission of nerve impulses. It is perfectly conceivable that the very fine nerve branches, which form the synapse, conduct impulses at a slower rate than do axon fibers. An experimental method, adequate for testing this idea, has not yet been found.

Reduced reflex time appears to vary with the comparative strength of stimulus. Very strong electrical stimulation of sensory nerves of the frog gives a reflex time no greater than it would be if a nerve fiber carried the impulse from the point of stimulation to the spinal cord and then directly to the muscle. Thus, strong stimuli are shown to pass through the neurones of the cord without any delay. Graduated strengths of stimulation even show a partial correspondence between the strength of stimulus and reduced reflex time.

The Correlating Action of Connecting Neurones.—Any one connecting neurone doubtless has synapses with many other neurones. Some of these and, in some cases, all of them are themselves connecting neurones. Excitation, travelling through the different branches of a connecting neurone, would be propagated over paths of different lengths and through different numbers of synapses before reaching motor neurones. Both the length of the path and the number of synapses in it are supposed to affect the time required for propagation. Different motor neurones are thus excited at different times. This is one reason why correlated action results from normal reflex stimulation. The synapses themselves doubtless behave differently at different times because a stimulus which is repeated very uniformly may call forth either a large or a small motor response in a given group of muscles under different circumstances. This

indicates that while some of the synapses, of certain connecting neurones are transmitting excitation, other synapses of the same neurones are not transmitting. The number of muscles brought into a correlated action is thus determined by the connecting neurones and their synapses.

Correlation partly depends on the nature of the stimulus. A repetition of the same stimulus at certain time intervals usually causes a progressive increase in the number of the responding muscles. After a sufficient number of repetitions, the extent of motor response decreases rapidly until it fails altogether. This indicates a fatigue somewhere in the reflex apparatus. Differing intensities of stimulus also cause corresponding differences in motor responses. For example, stimulation of the toe of a reflex frog by dipping into varying strengths of dilute sulphuric acid causes responses which vary in extent and brevity of reflex time with the strength of the acid used. This result may be due to stimulation of a progressively larger number of sense receptors within a given time as the strength of acid is increased. All these effects are shown to be due, in part at least, to the varying behavior of neurones because they are all markedly influenced by the condition of the spinal cord at the time of reflex stimulation.

Effects of the Condition of the Spinal Cord upon Reflexes.—The effects of repeated uniform stimulation suggest that hydrogen-ion concentration of some of the structures in the spinal cord has a relation to the conduction of excitation because these effects resemble the treppe and the fatigue phenomena of muscles. Further investigation of this apparent relationship is needed. The presence of certain abnormal substances can produce marked changes in the behavior of the spinal cord. Of these substances, strychnine produces the most startling results. A reflex frog, injected with a very small quantity of a strychnine salt, quickly becomes hypersensitive to reflex stimuli. A light touch which would ordinarily produce little or no response now causes extensive muscular contractions. As the action of the drug progresses, the motor responses to stimuli are no longer coordinated ones, but become convulsive and involve tetanus of large groups of muscles. The entire body of the frog goes into a stiffened condition because all muscles are tetanized simultaneously. The limbs are thus extended because extensor muscles are more powerful than flexor ones. Such spasms are brought on, under these circumstances, by the slightest imaginable stimuli. Vibration, caused by the slamming of a distant door, the slightest breath of air upon the skin of the animal or any other very slight disturbance is sufficient to bring on intense muscular spasms. The toxin produced by the tetanus bacillus has the same effect as strychnine and the name lock-jaw, commonly applied to this disease, when it occurs in human beings, refers to the uncontrollable stiffened condition of the jaw muscles as well as all other skeletal muscles. They go into spasms in response to very slight stimuli. The strychnine

effect upon reflex responses in the spinal frog is exerted upon the spinal cord rather than upon other structures in the neuro-muscular mechanism. This is shown by the fact that all spasms cease in the strychnine-poisoned frog when the spinal cord is destroyed by pithing. An interpretation of the strychnine and tetanus-toxin effects is usually given in terms of action upon synapses. These foreign substances are supposed to act specifically upon the resistance of synapses to the propagation of excitation. According to this theory, all synapses in the spinal cord of the poisoned animal transmit freely so that a very slight sensory stimulus results in distribution of excitation to practically every skeletal muscle in the body. Human beings, suffering from strychnine poisoning or tetanus infection, experience not only the violent pain of uncontrollable muscle cramps but also an extreme mental agony. Any effort of the will, even those exerted in an attempt to stop the violent tetanic contractions only brings on spasms worse than ever. Even inhibition has thus been turned into excitation and even the slightest imaginable stimuli cause violent responses. The entire mechanism of the central nervous system has lost all power of coordination and inhibition of muscular responses. The central adjustor mechanism is wrecked.

Chloroform antagonizes the strychnine and tetanus-toxin effects. The muscular spasms can be entirely stopped by the proper depth of chloroform anaesthesia. Although the fatal result of lethal doses of strychnine and of tetanus infection cannot be long postponed by the use of chloroform, its antagonism to the specific effects of the poisons upon the spinal cord is clearly shown. The inference is that if strychnine decreases synaptic resistance to transmission, chloroform increases this resistance. Whether or not this theoretical explanation is correct, the phenomena shown by the action of these drugs cannot be interpreted in any way that does not take into account the fact that conduction from neurone to neurone is subject to great changes. The hypothesis of changing behavior of the synapses accounts for this fact in a satisfactory way.

Normal Graded Synaptic Resistance.—The variations of reflexes, in accordance with the conditions of artificial stimulation and the condition of the spinal cord, doubtless have their counterpart in the variations of the normal behavior of reflexes. The groups of muscles that respond to reflex excitation are normally coordinated in their action only so far as individual muscles are excited to just the proper extent. As muscles are normally coordinated in reflex acts, the nervous paths leading to motor neurones appear to be in such a condition, under ordinary circumstances, that some of them are more open than others. This idea is sometimes expressed by saying that the nervous system possesses graded synaptic resistance. Comparatively weak excitation is conducted through the more open paths whose synapses are less resistant. Stronger excitations are also conducted through the more resistant paths. It is

important to remember in this connection that in accordance with the "all or nothing" principle, the term, stronger stimuli, merely means an increased number of stimuli, produced either by the excitation of a larger number of sensory nerve fibers or by an increased frequency of stimuli in the same numbers of fibers. How an increase in the number or the frequency of the stimuli entering the reflex mechanism can overcome synaptic resistance is not known.

Certain normal conditions other than increased strength of stimulus also cause increase of response. One of these conditions is the simultaneous excitation of a reflex arc other than the one to be opened up. This is shown by the phenomenon called *reinforcement of the knee-kick*. If the subject of a knee-kick test clenches the fist or makes any other voluntary muscular movement at the same time that the stimulating blow is struck upon the knee ligament, the muscular response of the knee-kick is larger than it would otherwise be. This is taken to indicate that activity in one part of the nervous system tends to break down synaptic resistance in other parts. This idea is further substantiated by the fact that the knee-kick varies in extent in direct proportion to the general mental activity and alertness of the subject. The extent of the knee-kick even shows a fairly regular daily variation according to observations on some individuals. Lombard concluded from his observations that, "the knee-jerk is increased and diminished by whatever increases and diminishes the activity of the central nervous system as a whole." The response disappears entirely during sleep, is comparatively small during conditions of mental sluggishness or repose and is conspicuously large during mental excitement or irritation.

According to the theory of synapses, normal behavior of the nervous system is dependent to a very large extent upon maintainance of graded synaptic resistance. Abnormally low synaptic resistance, in part or all of the nervous system, results in useless and uncontrolled activity. The common phrase, "nervous person," refers to one who responds too freely to stimuli. He is apt to jump at a slight sound; his movements are restless, almost unceasing and often useless. The stream of sensory impulses, in such a person, plays with little restraint upon the motor neurones because many reflex paths lack their normal resistance.

Habits as Developed Reflexes.—The common expression, "act by force of habit," implies the mechanical nature of activities that are habitual; for the words suggest that one can respond to a given set of conditions in only one way. But acts which eventually become habitual are not done in this seemingly mechanical way at first. The application of automobile brakes at the right time requires careful thought and fixed attention on the part of a beginning driver; but after driving becomes habitual to him, any circumstances, which necessitate stopping, result in application of the brakes by the driver, even though his attention appears

to be fixed on other matters and the habitual driver is very apt to find himself involuntarily making the motions of putting on the brake, when someone else is driving the automobile. Training appears to make one react automatically. Very many illustrations could be given. All skilled actions, handling of machinery and tools, playing of musical instruments, making the movements required in any skillful athletic game and the ordinary acts that are daily performed by human beings, come to be comparatively automatic. The movements for any such acts, at first slow and awkward, become easier and easier with repetition until they are finally done with no apparent attention. "Practice makes perfect." Even such common acts as standing erect and walking are really habits which must be slowly and difficultly acquired by the child. The power to acquire habits is of great value in human life. Most of our everyday acts are performed without the need of special attention. We do them in a reflex manner. If this were not the case, mental activity would need to be concentrated continually upon acts that are necessary for mere existence; but acquisition of new reflexes gives the mind a certain amount of freedom to attend to the gaining of new knowledge. Human progress is thus primarily dependent upon the ability to acquire habits. From the standpoint of physiology, habits may be defined as acts which are done at first in a typically voluntary way, but which after sufficient repetition, are done in a comparatively reflex way. A physiological explanation of the acquisition of habits is based on the assumption that habit-forming involves the establishment of new reflex paths. The resulting arcs, however, are not composed of new neurones, for a vertebrate is born with the same number of neurones that it possesses throughout life. The new paths are not built but merely become functional for reflex acts. Repeated use of certain neurones appears to wear a path across them. According to the theory of synapses, the paths of conduction between certain receptors and effectors are at first partially blocked and can only slowly become comparatively open as a result of repeated voluntary use. The explanation also assumes that numerous other possible paths, which should be blocked to further the efficient performance of the given act, are at first comparatively open. This part of the explanation is designed to account for the fact that many muscles are used unnecessarily during the first awkward attempts of a person who is learning how to perform a skilled act. His coordination is imperfectly adjusted for the given performance. With acquisition of skill, the paths, conducting excitation unnecessarily, are blocked so that a skilled person does not use his muscles inefficiently. This is the chief reason why a skilled worker is less fatigued by the performance of a given amount of work than is a "green hand." The acquisition of skill or of any habit means the development of special paths of least resistance for the conduction of excitation through the central nervous system. These

acquired reflex paths are due, according to the theory of synapses, to new adjustments of graded synaptic resistance.

Inhibitions in the nervous system involve blocking of certain paths of conduction, so that sensory impulses are not permitted to reach motor neurones. This effect is very largely exerted by the action of neurones in the brain upon connecting neurones which lie in the direct path of reflexes and constitute parts of reflex arcs. Such inhibitions play an important rôle not only in the development of the skill which is dependent upon ability to act reflexly, but also in the formation of the habits which constitute the manners and customs of human intercourse. Inhibition is, in short, the physiological aspect of self-control.

One of the marked distinctions between man and other vertebrates is the comparatively great ability of man for acquiring and developing habits. Man can acquire habits more freely than other vertebrates can and he is also able to develop some of his habits into a better state of reflex perfection. This is the basis of the skill of the human hand. At the outset of life, however, a human being is seriously handicapped as compared with some other vertebrates. He is born with comparatively few reflex arcs established and ready for use. The sucking reflex, the swallowing reflex, the winking reflex and many reflexes governing non-striated muscles are among those that are functionally complete in the new-born child. But the reflex arcs that later on give coordinated control of most of the skeletal muscles are not in functional order in the infant. This is in marked contrast to the reflex apparatus of a new-born calf, which can perform the highly coordinated acts of standing and running. Even kittens and puppies can coordinate sufficiently to crawl, shortly after birth, even though their still unopened eyes make them appear less advanced in their state of nervous organization than the new-born child. But the very disorganization of the nervous system of the child leaves it all the more plastic, so that its future development can provide reflex arcs adapted to further the performance of the many acts which make up the habits of the human individual and enable him to adapt himself to his environment in ways that are not available to any other animal.

Memory in Relation to Reflexes.—In the early stages of the acquisition of any habit, memory is an indisputable accompaniment of the process. But memory appears to be less concerned in the fixing of some habits. We tacitly acknowledge the varying effects of memory upon reflexes when we say of one skilled act that we are "out of practice" for its performances; while of another act we say we have "forgotten how" to do it. With the fading of memory, certain of the connections which establish reflex arcs appear to fail. In other cases, they are not so much affected. The skill, which gives efficiency in the trades, the arts, and in sports, may be almost lost as a result of disuse. Walking, skating and swimming, on the other hand, are accomplishments which are said to be

never lost after they are once acquired. Certainly some acquired reflexes are much more easily reacquired after disuse than are others. In any case one does not necessarily remember, in the ordinary sense, the experiences which he had at the time he acquired any given reflex. One learns to walk, for example, without any permanent memory of the learning process. On the other hand, our dependence upon memory is sometimes greater than we realize. The following case serves as an illustration. A middle-aged man became totally blind. A few months later he resumed his habit of shaving himself. He was able to do this just as efficiently as when he had his sight, but only if he felt perfectly sure that a mirror was in front of him. Although he laughed at the absurdity of it, he nevertheless found that any attempt to shave without the mirror threw him into confusion. Apparently he reconstructed from memory the mirrored image of the movements of his hand in guiding the razor.

The very noticeable *rôle of inhibition* in the developed reflexes shows that the stored up effects of experience are in some way required for efficient performance of this kind of reflex. The persistence of these inhibitions varies in different species. This is shown in experiments in which an animal has learned to find its way to food through a maze. After it has found, by experience, how to avoid the wrong turnings in the maze, persistence of memory can be measured. The cockroach "remembers" its way through the maze only about a half an hour. This is an extreme case. Higher animals retain the impression of a habit much longer. In the vertebrates, memory depends entirely on the brain. Any injury to the cerebrum tends to destroy ability to profit by experience and decerebration completely destroys this ability. The general inference from comparative physiology is that developed reflexes are dependent upon the establishment of reflex arcs which involve neurones in the brain. The specific usefulness of these arcs depends partly upon the proper adjustment of graded synaptic resistance, but also depends in part upon processes that we call memory. The latter can be interpreted in terms of biophysics and biochemistry as a slowly reversible physico-chemical change in neurones of the brain.

Tonic Activity of the Spinal Cord.—Sensory impulses are constantly playing upon the spinal cord and to some extent upon the brain. The result is a sustained reflex activity except in so far as inhibitions interrupt paths of conduction to motor neurones. This is shown by the sustained contractions of skeletal muscles. Some of them are always in a partly contracted state or tonus except during deep sleep. This sustained contraction is not due to an inherent property of skeletal muscles themselves, for it entirely disappears after section of motor nerves and is thus shown to be the result of reflex excitation. This sustained activity of spinal reflex arcs can be more clearly shown, especially in vertebrates, when the controlling influences of the brain upon the spinal cord are

removed. This result is obtained by decerebration. After this operation, cats and dogs show what is called **decerebrate rigidity**. The muscles of their limbs are in a state of partial sustained contraction owing to reflex tonus. The explanation of decerebrate rigidity is that removal of the cerebrum stops the inhibitions which were previously being exerted by the brain centers upon the spinal motor neurones. The spinal reflex arcs are thus opened so that sensory impulses result in continuous muscular responses.

The inhibitory effects of the brain upon reflex arcs is probably much less extensive in a frog than it is in a mammal. At any rate, the cessation of these effects, after pithing of the brain, does not produce decerebrate rigidity in the frog. This animal is normally more lethargic than a mammal and correspondingly, the reflex frog exhibits very little evidence of tonic activity in the spinal cord.

Reciprocal Innervation.—Excitation of certain muscles is accompanied by simultaneous inhibition of their antagonists. In invertebrates, this is accomplished, in certain cases at least, by two sets of nerves which have different effects of the muscle—one set excites, the other inhibits. This arrangement is called reciprocal innervation. For the skeletal muscles of vertebrates, the arrangement of nerves is very different (p. 293). Dual control is not obtained in these muscles by a double innervation. All efferent nerves to skeletal muscles are excitatory, none of them inhibitory. Yet dual control is just as apparent in the movements of vertebrates as it is in those of other animals. Walking, for example, necessitates not only excitation of muscles but also their inhibition. Excitation of flexors must be accompanied by inhibition of extensors and vice versa. In walking, these two effects alternate with regular rhythm in nearly every muscle of the legs.

The mechanism securing this dual control of vertebrate muscles is in the central nervous system. It is, nevertheless, called reciprocal innervation. It has been extensively investigated, chiefly by Sherrington. The most satisfactory studies are those made upon the decerebrate cat. Inasmuch as tonic activity of the spinal cord is released from inhibition in this preparation, all the leg muscles are in a partially contracted state. Under these circumstances, stimulation of a sensory nerve, which causes contraction of the flexors, results in a simultaneous relaxation of extensors. (Fig. 92.) Suitable electrical excitation of sensory nerves of both legs can produce regular movements in which excitation and inhibition occur rhythmically and are so coordinated that contraction of any group of muscles is simultaneous with relaxation of their antagonists. One of these movements is called the "stepping reflex." It involves muscular contractions similar to those of walking. Such effects can be obtained by stimulation of afferent nerves only, so that what appears to be muscular inhibition is really inhibition of motor neurones. Stimulation of one

sensory nerve excites certain motor neurones and simultaneously inhibits others which were being excited to some extent before the stimulus occurred. The particular motor neurones which are thus inhibited, are those controlling the muscles that are antagonistic to the specifically excited ones.

A mechanism which opens some conducting paths in the spinal cord and at the same time closes others, is difficult to picture. Indeed, the



FIG. 92.—Reflex inhibition. A muscle of the leg was made to contract by stimulation of the skin of the foot on the opposite side. At the point shown by the upper of the two signal lines, the foot on the same side as the muscle was also stimulated. The contraction is abolished because of inhibition exerted in the central nervous system. (*Sherrington.*)

theory of synapses has not been definitely proved to apply to the explanation of this dual control. A sensory impulse, upon entering the spinal cord, excites connecting neurones which have numerous different branches. Some of these branches transmit excitation to motor neurones which are thus excited; other branches transmit excitation to other motor neurones which are thereby inhibited. The actual inhibition need not necessarily occur in those motor neurones which are directly connected to muscles; but may take place in what are sometimes called **premotor neurones**. These are supposed to command excitation of more than one motor neurone. A premotor neurone is, then, a kind of motor center and although it serves as a connecting neurone, its excitation is equivalent, presumably, to excitation of motor neurones. Conversely, its inhibition is equivalent to inhibition of motor neurones. The experimental fact of dual control

of skeletal muscles shows that, in some way, the premotor neurones or the motor neurones or both of these structures must be subject to both excitation and inhibition. An explanation of this dual control is naturally sought in terms of the general theory of the difference between excitation and inhibition. This difference, as explained above, depends, in the case of muscle cells, upon the place or mode of ending of the nerves which respectively cause excitation and inhibition. In the same way, dual control of a neurone has been supposed, by some writers, to be determined in accordance with the path through which excitation enters it. But inhibition of motor neurones can also be explained by means of the behavior of synapses. According to this idea, inhibition of a neurone is merely a cessation of its excitation and is caused by a block in the conducting power of the synapses through which excitation must reach the neurone. Such a block could be produced, conceivably, by a change in the number of impulses reaching a synapse per unit time. If this synapse were so placed, with respect to premotor or motor neurones, that it formed a part of the final common path, the block would inhibit motor responses. Such a change in the rate of impingement of impulses upon a synapse could occur in either or both of two ways: (1) Arrival of impulses over a different number of connecting paths, (2) a change in the rate of transmission of impulses over a given number of paths. The belief that a change in the rate of impingement of impulses can so block a synapse as to change excitation of its neurone into absence of excitation (inhibition)—this belief is based chiefly upon the interpretation of the Wedensky phenomenon in which transmission of nerve impulses across the myo-neural junction is completely blocked for a certain rate of stimulation of the nerve although effective transmission freely occurs when a slower rate of excitation is used.

Automatic Reciprocal Innervation.—The reciprocal innervation, which controls flexors and extensors of the legs, differs from similar innervations in that the alternate excitation and inhibition of their muscles, sometimes occurs in the decerebrate animal during the absence of all artificial afferent stimulation. This behavior suggests that the neuro-muscular mechanism, consisting of these two groups of muscles and their immediately connected neurones, is automatic or, in other words, is intrinsically self-controlled under certain experimental conditions.

A tentative explanation of this behavior is based upon the probable effects of two kinds of nerve fibers that constitute parts of the mechanism. One of these sets consists of side branches of the axon fibers of the motor neurones. Each axon appears to have one branch coming off near the point where the axon crosses the border line between gray and white matter of the spinal cord. The termination of this side branch is not definitely known; but the idea that it forms a synapse with a motor neurone has been generally favored. If it forms such a synapse, the side

branch of each extensor motor neurone might conceivably connect with a flexor motor neurone or a flexor premotor neurone and correspondingly, the side branch of each flexor motor neurone might connect with the extensor motor apparatus. An impulse, travelling along the axon fiber of any one of these motor neurones, is bound to be propagated also along the side branch. If the branch transmits impulses through a synapse to an antagonistic motor neurone, the latter may be thereby inhibited. None of the known facts of neurone physiology contradict this possibility. Such a mechanism would account very well for the observed fact that the reciprocal relations between flexors and extensors is automatic, so that excitation of one of these groups of muscles tends to produce inhibition of the other group.

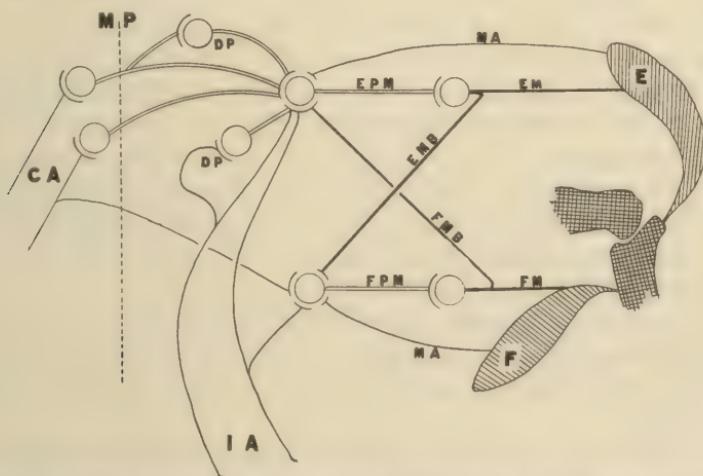


FIG. 93.—Diagram to illustrate a provisional explanation of reciprocal innervation of antagonistic muscles. *E*, extensor. *F*, flexor. *MP*, median plane of the body. *IA*, ipsilateral, afferent neurone. *CA*, contralateral, afferent neurone. *DP*, delay path to account for after discharge. *EPM*, extensor premotor neurone. *FPM*, flexor premotor neurone; *MA*, afferent neurone from muscle. *EM*, extensor motor neurone. *FM*, flexor motor neurone. *EMB* and *FMB*, extensor and flexor motor branches. (From Forbes, *Physiol. Rev.*)

The second set of nerve fibers, supposed to be concerned in the autonomy of the flexor-extensor mechanism, is the group of sensory neurone fibers which convey impulses from the muscles themselves to the spinal cord. A discussion of these structures will be taken up later. For the present, it is sufficient to state that contraction of the muscle excites these nerves and therefore causes reflex excitation of motor neurones.

A hypothetical arrangement of this set of fibers and also of the set described above is shown diagrammatically in Fig. 93. Although this scheme, as suggested by Forbes, is not in disagreement with any known facts, it still requires, as he has carefully pointed out, further histological and physiological investigation before it can be regarded as anything

more than a working hypothesis. Even if it presents only a little of the truth, it at least helps one to understand the autonomous dual control of certain reflexes. The control may be imagined to work in the following way. Any stimulus, which instigates contractions of flexor muscles, simultaneously inhibits extensors. The flexor muscles, by contracting, further stimulate themselves reflexly until the succession of impulses, thus brought to bear upon the motor neurones, produces a condition of fatigue in the synapses which have been actively transmitting. Such a fatigue might easily result from a slight and localized increase in hydrogen ion concentration. If this were sufficient to block the transmission of impulses through the previously active synapses, contraction of flexor muscles would cease and simultaneously, inhibition of extensors would also cease. The latter effect would be produced by removal of the block upon synapses of extensor motor neurones. Thereupon, they would freely transmit impulses still coming to them from the flexor muscles through connecting neurones. These impulses would cause contraction of extensors and a simultaneous relaxation of the remaining tonus in flexors. Contractions of the extensors would produce, in due order, a fatigue in the synapses of their motor neurones and thereby bring about their relaxation and permit flexors to contract again. This whole cycle of events would thus produce alternating but correlated activity of opposing muscles in an automatic way.

The true reciprocal innervation, which gives a dual control of the muscles of invertebrates, occurs to only a limited extent in the vertebrates. The dual control of the heart and of visceral muscles, as described above, is of this type.

For the most part, however, inhibition and consequent dual control is provided for, in higher animals, by the type of reciprocal innervation which is entirely contained in the central nervous system. The extent to which the inhibitions are exerted upon connecting neurones as contrasted with direct effects upon motor neurones is not yet known. Although the description given here applies more particularly to the mechanism of reciprocal innervation as it occurs in the spinal cord, a similar dual control also governs the contractions of muscles which, like those that move the eyeballs, are innervated by cranial nerves. Reciprocal innervation in the brain may be of the same general type as that in the spinal cord.

After-discharge.—When sensory nerves are stimulated in the spinal animal, the resulting response persists after stimulation has ceased. This phenomenon is called the after-discharge. It lasts in some experiments as long as 12 sec. and is always longer than it would be if it were merely due to the time required for transmission of the last excitation given to the receptor, provided the excitation passed through the simple reflex arc to the muscles. In other words, the after-discharge is longer

than the duration of reflex time. The after-discharge is promptly checked, however, by any suitable inhibitory stimulus. For example, the after-discharge contractions of extensor muscles are instantly abolished by stimulation of nerves which cause the contraction of the opposing flexors. In ordinary voluntary movements, undue prolongation of contraction is doubtless prevented either by the inhibitions which brain centers exert upon motor neurones or by other inhibitory influences.

The cause of the phenomenon of after-discharge is indicated by the fact that it is greatly decreased, in the case of certain spinal reflexes, by transsection of the spinal cord at a point above the level of the location of the arc necessary for the reflex. The theoretical explanation, which has been suggested, depends upon the fact that the numerous collateral paths of a reflex arc are some of them longer and more complex than others. By way of the shorter paths of the arc, conduction of excitation requires only the comparatively brief period of reflex time. But by way of the longest of the paths, conduction of excitation requires much more time. This explanation appears all the more probable in view of the delay of the propagation of impulses at each one of the comparatively large number of synapses occurring in the longer and more complex paths.

The Conditioned Reflex.—As explained above, some responses, which closely resemble reflexes, cannot be obtained unless conscious attention is directed upon the stimulus. Such reactions are called psychic reflexes. Pawlow has found a method for investigating them. His chief investigations were made upon the reflex secretion of saliva in the dog. The fact that this response may be a psychic reflex is shown by the observation that a dog, which is fed by only one person, will secrete saliva upon seeing this person. This response occurs in the hungry animal even when it can neither see nor smell any food. The animal has evidently acquired a reflex. The stimulus for it is the sight of a certain person. The response is the secretion of saliva. This acquired reflex is conditioned, however, upon a memory association which connects the presence of a certain person with the eating of food. Pawlow, therefore, calls this kind of reaction a conditioned reflex. For the finer analysis of this phenomenon, Pawlow used animals that had been operated on to make a salivary fistula, an artificial opening from the salivary duct to the outside of the jaw. A tube, inserted in the fistula, was connected with a delicate indicator, which could be watched by an observer, out of sight of the dog. The number of drops of saliva secreted could be accurately measured. Each dog was kept under carefully controlled conditions which eliminated all accidental stimuli such as sounds and the changing of lights or temperatures. Food was given under conditions which varied only in a known way. In some of the experiments, a dog received food while hearing a musical note of a certain definite pitch. In due time, the animal had a conditioned reflex by which secretion of

saliva was produced upon hearing this note, even though no food was presented. The discrimination between notes, which would give the response and those which would not, was surprisingly fine. For example, an animal with a conditioned reflex for a note of 100 vibrations per second would not respond to a note of 104 vibrations or to one of 96 vibrations.

In some cases, conditioned reflexes were established by giving food to the dog at a certain definite time after the other stimulus was given. For example, food was given, in one experiment, two minutes after the ringing of a bell. In due time, the animal had a conditioned reflex which caused the secretion of saliva after the ringing of the bell, but always *exactly two minutes later*, irrespective of whether food was or was not then given. This is a delayed reflex. The nervous system behaves, in this reaction, as though it were able to keep time. The well known ability of some individuals to waken at a certain time, if they go to sleep with the expectation of doing so, may be a delayed reflex.

A sound, not associated with food, could bring on secretion of saliva in the dog after the ringing of the bell and before the end of two minutes. This observation indicates that some active process occurs in the nervous system after the sound of the bell, but that this process includes an inhibition of the neurones which control salivary secretion. This inhibition appears to be released by the sound which, of itself, would otherwise call forth no response. Pawlow showed by other experiments, that inhibitions do actually play a part in conditioned reflexes. He even produced a conditioned reflex for the inhibition of salivary secretion rather than for excitation. A dog was given food during the ringing of a bell; at other times, the same animal was fed during the flashing of a light. At still other times, bell ringing and light flashing occurred together, but on these occasions the animal never received food. In due time, the conditioned reflexes for either ringing or flashing alone were developed, while the two stimuli together were ineffective. Moreover, secretion, started by ringing of the bell, was instantly stopped when the light began to flash. The reverse of this experiment was also effective, that is, secretion in response to the light was inhibited if the bell began to ring.

The rôle of inhibition in conditioned reflexes is probably a very important one. Pawlow showed that a dog was very apt to fall asleep if the stimulus for the conditioned reflex was not followed by the giving of food. This fact suggests that while the stimulus was acting, it inhibited large numbers of neurones. According to Pawlow's interpretation, this inhibition is sufficient to bring on sleep in the absence of further counteracting stimuli. This idea is in accord with the familiar fact that a person is very apt to fall asleep when he fixes attention upon a lecture, a book, a picture or anything which is allowed to inhibit consciousness of other stimuli, but which does not itself arouse an effective emotional or other response. The sound of the droning voice or the sight of the unin-

teresting printed page becomes an inhibitory stimulus if it holds sway over the central nervous system. Moreover, a person can develop what appears to be a conditioned reflex for sleep in response to a specific stimulus. Some persons are so fortunate as to acquire the power to fall asleep quickly as a result of merely lying down and closing the eyes. They are not apt to be troubled with insomnia. Other persons are so unfortunate as to have become dependent upon some particular stimulus, such as reading, as a sleep inducer. Probably very many of our developed reflexes are conditioned in a more or less definite way and inasmuch as all complex reflexes involve inhibition of a considerable number of neurones, the theory that a stimulus may inhibit all the neurones concerned with consciousness and so cause sleep, is not an impossible one. A study of conditioned reflex inhibitions might possibly help to explain hypnotism.

Pawlow obtained evidence showing that the consciousness of pain may be inhibited when a painful stimulus is used to develop a conditioned reflex. For example, an electrical stimulus, known to be painful to a human being, was given to a certain spot on a dog's leg every time the animal received food. In due time, this particular stimulus caused the secretion of saliva in the absence of food. After this conditioned reflex was thus developed, the animal showed no signs of pain during this stimulation, although the same electrical current, through the same electrodes, applied to the skin only 1 cm. distant from the usual location, caused signs of pain and did not arouse any salivary secretion. Evidently a specific group of sensory neurones is a necessary part of the arc of the conditioned reflex and the inhibition of sensation can be obtained only when the stimulus excites these particular neurones. One interpretation of these experiments is that paths, which once connected these particular sensory neurones to centers for consciousness of pain, have become less open to conduction of excitation than are paths connecting these same sensory neurones to the ones that excite salivary secretion.

All conditioned reflexes must depend upon associations formed in the cerebrum. Operative injury to a certain part of the cerebrum of a dog with a conditioned reflex entirely destroyed the ability of the animal to respond to the stimulus, although a similar operation on a different part of the cerebrum of a control dog did not destroy its conditioned reflex. The locality of the cerebrum which was thus shown to contain the mechanism for the conditioned reflex, was, in every case, a part known to be in physiological connection with the receptors used for developing reflexes. Complete decerebration destroys all conditioned reflexes.

Unfortunately, Pawlow has not been able to continue and extend these experiments. Their interest and importance warrants further investigation. The facilities and resources of the Imperial Institute of St. Petersburg were required to make possible these expensive and difficult researches.

Tropisms.—When a sessile animal or a fixed plant bends or grows in a definite direction in response to a definite stimulus, turning, for example, toward the sun, the movement is called a tropism. This term has been extended to include the definite oriented movements of motile organisms. If a plant or animal turns or moves toward the source of the stimulus, it is said to show *positive tropism*; if it turns or moves away from the source of the stimulus, it is said to show *negative tropism*. Positive **heliotropism** or **phototropism** is the tendency to turn or move toward light. Positive **galvanotropism** is the tendency to turn or move toward the positive pole (anode) when in the stream of an electric current. Positive **geotropism** is the tendency of the roots of a plant or of parts of animals to grow or to bend downward under the influence of gravity. Similar upward growth or bending is negative geotropism. Positive **chemotropism** is the tendency of an organism to turn or move toward the source of a given chemical substance which is diffusing from its source into the surrounding medium. **Stereotropism** is the tendency of an organism to orient itself in a certain definite way with respect to solid bodies. Numerous other tropisms have been described, for example, **rheotropism** or orientation with respect to stream flow and **thermotropism** or orientation with respect to a source of radiating heat in the environment; but as many of these reactions can be shown to be due to other tropisms or to behavior which is not typical of a tropism, they will not be discussed here. The terms, phototaxis, chemotaxis, etc., are sometimes used with the same meanings as those of the corresponding tropisms.

Tropisms afford an explanation for many aspects of animal behavior, which the physiology of reflexes alone cannot explain. In a certain sense, a tropism is itself a reflex when it occurs in an animal with a central nervous system, but differs from a reflex in that it involves the coordinated action of so many reflex arcs that it may be regarded as a reaction of the organism as a whole. A reflex, on the other hand, need not necessarily involve as many different reflex arcs as a tropism, so that only a segment or small portion of an animal responds.

The Theory of Tropisms.—Loeb has formulated a general theory of the nature of tropisms which explains many of the reactions of higher animals as well as the more obviously tropistic reactions of plants and lower animals. The basis of this theory is the symmetry of the structure of organisms. Two kinds of symmetry are recognized, namely, radial and bilateral. Radial symmetry is possessed by most plants and by numerous animals, for example starfishes. Bilateral symmetry occurs in the majority of animal species. The behavior of a bilaterally symmetrical animal will serve to illustrate the theory of tropisms. Suppose a stimulus, such as light, comes from a source directly in front of such an animal and on the line of axis of its symmetry. Any receptors that are excited by the stimulus, are symmetrically located on either side of the animal

and those correspondingly located on opposite sides are excited to the same degree. The symmetrical arrangement of neurones in the organism insures such a conduction of excitation from the receptors to muscles, that symmetrical muscles are put under equal tension. This insures that, if the animal moves forward, it is bound to move in as straight a line as the slight imperfections in its bilateral symmetry permit. In short, it goes straight toward the source of the stimulus. If, however, the source of the stimulus is not in line with the axis of symmetry of the animal, symmetrically opposite receptors will not be excited to an equal extent. Consequently the tension of symmetrical muscles will not be equal and the greater tension developed on the side which is more strongly excited will turn the animal until its axis of symmetry is in the position which gives equal excitation of opposite receptors. Muscular tension is then bilaterally symmetrical and the animal therefore moves in a straight line. Due allowance has to be given to the crossing over of reflex paths, a condition which gives the receptors of one side an opportunity to excite certain muscles of the opposite side.

A few asymmetrical animals, mostly protozoa, are known. The flat-fishes, such as the halibut, flounder and sole, have the two eyes on one side of the body, so that they furnish examples of asymmetry even among vertebrates. Inasmuch as animals, made asymmetrical by experimental procedures, such as covering of one eye, behave in a manner which agrees with the theory of tropisms, it seems probable that any tropistic behavior of naturally asymmetrical animals is also in agreement with the theory, provided due allowance is made for their structure.

Forced Movements.—The theory of tropisms is partly based upon what are called forced movements. These occur after certain injuries to the brain. If the left half of the cortex of a dog's brain is injured so as to interrupt certain of its functional connections, the animal tends to walk in circles always turning to the left. Correspondingly, a similar injury to the right side of the brain results in turning motions to the right. This is explained by the fact that neurones on one side of the brain form parts of the reflex arcs which excite muscles of the *opposite* side. An injury to neurones of the left side therefore causes a diminished control of muscles of the right side of the body with poorer coordination and less tension in these muscles than in those of the left side. There is, indeed, a partial paralysis of the muscles of the right side, so that it becomes very difficult for the animal to turn to the right; while the normal tension of muscles on its left side tends to make it turn to the left. Very similar conditions occur in human beings suffering from certain diseases or brain injuries. As such individuals turn the body, or fall over, in a certain direction in spite of a will to the contrary, these reactions are called forced movements. They have been observed in many different species of animals. The reactions of a dragon fly larva, after removal of one or

both of its cerebral ganglia, illustrate forced movements (Fig. 94). In this animal the muscles of each side of its body are excited through arcs in the cerebral ganglion of the same side.

Inasmuch as forced movements result from a destruction of the bilateral symmetry of the nervous system of an animal, they indicate that the similar normal tropistic movements are the result of asymmetrical

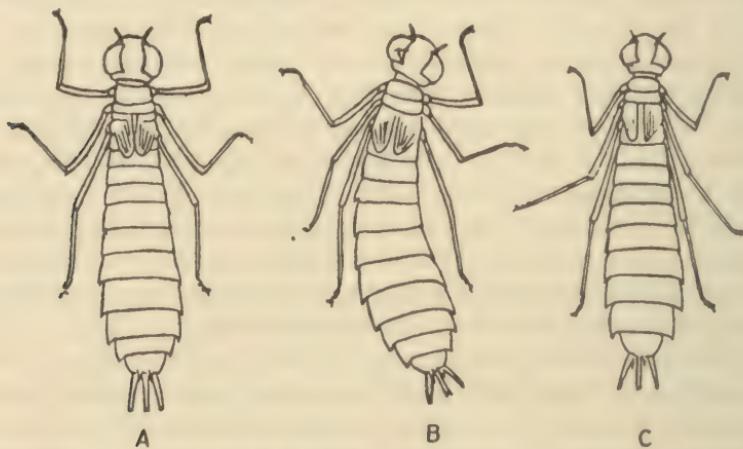


FIG. 94.—Forced movements of the dragon fly. *A*, normal position of the body. *B*, position after removal of the left cerebral ganglion. *C*, position after removal of both cerebral ganglia. (From Loeb's "Tropisms and Animal Conduct," J. B. Lippincott Co.)

stimulation of an animal with a symmetrical nervous system. In a sense, then, a tropism is a forced movement, but occurs only under certain conditions of stimulation; while a forced movement, in the sense usually meant by that term, occurs in response to many conditions of excitation, because of a permanent injury to the nervous system.

Galvanotropism.—Many different species of animals have been found to show galvanotropism. This reaction was especially investigated by Loeb and his co-workers. The shrimp, *Palaemonetes*, was one of the species successfully used. If a shrimp is placed in a trough, the ends of which are electrodes connected with a battery and if a current of the correct intensity is passed through the water in the trough, the animal tends to move to the anode. If the animal is placed with its head toward the anode end of the trough, it either swims or crawls forward. If its tail is toward the anode, it swims or crawls backward. It can also crawl sidewise and if it does this under the influence of the electric current, it moves toward the anode. Loeb has shown that all these movements are forced in the animal because of the relative tension of flexor and extensor muscles which move the tail when the animal swims and those which move the legs when it crawls. Similar changes in the relative tension of different muscles cause forced movements of other crustacea, of tadpoles,

of salamanders and of numerous other animals under the influence of the electric current. A phenomenon, similar to galvanotropism and doubtless due to the same mechanism, can be observed in human beings. When an electric current is passed through the head, a person is very apt to fall down and always tends to fall toward the side of the anode; that is, if the anode is on the left side of the head, the person falls over toward his left.

An explanation of galvanotropism is based on the phenomena of electrotonus (p. 267). Loeb points out that just as the constant electric current causes a heightened excitability at the cathode of a nerve and lowered excitability at its anode, so a similar electrotonus may be supposed to exist in a neurone or chain of neurones when traversed by the current. A given neurone might readily be so located in an animal that during a certain orientation with respect to the electric current, this neurone would be rendered hyperexcitable; but during a different orientation, the same neurone might be rendered comparatively inexcitable. If all the neurones, which control flexors and extensors of the shrimp are located as Loeb assumes they are, a certain group of flexors are excited while the opposing extensors are inhibited. In other groups of opposing muscles, the reverse conditions occur. The animal is thus forced to move in certain definite ways. If this theory correctly explains galvanotropism in the shrimp, certain special arrangements of its motor neurones must be assumed because this animal responds to the current in the complex ways described above; but such evidence as is available to show the arrangement of the nervous system in this animal and in other crustacea is in agreement with the theory.

Heliotropism.—The most familiar example of heliotropism is the turning of plants toward the sun. The same type of response also occurs in numerous sessile animals. It has been observed in several varieties of hydroids. The responses of the sea-anemone to light (p. 338) are of the same character. Some of the tube worms so respond to light that they not only turn the soft parts of the body, but, in due time, bend their hard tubes by means of the secretion of new material onto the inside of the tube. Heliotropism has been observed, in all classes of animals from protozoa to mammals. It shows to a remarkable degree the underlying principle of tropisms because, in their responses to light, plants and animals can be shown definitely to move in a forced way, when light causes an unequal excitation of corresponding photo-sensitive parts (receptors) of a symmetrical organism.

Heliotropism has been studied most extensively in the insects, of which very many species show a tropistic behavior toward light during at least a part of their life history. Many of them are positively heliotropic, others negatively and some respond positively at some stages of their life histories and negatively at others. The behavior of certain

species of flies is instructive. When one eye is experimentally injured or is covered over with a light proof coat of black paint, positively heliotropic flies tend to turn in circles. If the right eye is blackened, they turn in circles toward the left and vice versa. This corresponds to the behavior of the normal animal which, in moving towards the light



FIG. 95.—Forced movement of the robber fly. With one eye covered with black paint it climbs spirally instead of vertically. (After Garrey, from Loeb's "Tropisms and Animal Conduct," J. B. Lippincott Co.)

but slightly deviating from the direct path of the light, receives more illumination of one eye than of the other. The animal then tends to turn until both eyes are equally illuminated. The case of the robber fly, investigated by Garrey, is of particular interest. This animal, like numerous other insects, tends to crawl upward upon vertical surfaces. This is due probably to the orienting effect of gravity upon the animal. The heliotropic response of such insects can overcome the tendency to move upward if the two tendencies are in opposition; but when the robber fly has had one eye blackened, it will no longer crawl upward upon a vertical stick but moves in spirals as indicated in Fig. 95. On a flat surface the animal has a tendency to move in circles with the normal eye toward the center. The muscles on the side of the blackened eye have a diminished tension, while those on the other side have their normally high tension because they receive excitation, due to the action of light on the photosensitive receptors of the normal eye. The tendency of many kinds

of insects to go towards the light is similarly explained by the muscle tension theory; for if they are moving toward the light, their two eyes are equally stimulated and the resulting equality of tension in all pairs of bilaterally symmetrical muscles keeps them moving toward the source of the light, while any circumstances tending to orient them, temporarily, away from the direction of the source of light are quickly counteracted by the resulting inequality of light stimulation of the two eyes and the consequent inequality of tension in pairs of bilaterally symmetrical muscles. Anything which might be called a purpose in the part of the insect or any results of experience or memory have nothing to do with this reaction. The well known tendency of the insect to fly into a flame, even after having been singed by it, shows, as Loeb suggests, that the animal is literally "the slave of the light."

Heliotropism moves an animal toward light just as mechanically as it does the so-called mechanical dog, invented by Hammond. This device consists of a box, containing certain electrical apparatus and mounted on three wheels. Two of the wheels are geared to an electric driving motor inside the box and the third is so arranged that it can be veered to either side by electromagnets symmetrically placed on either side of its bearings. Two condensing lenses, on the front of the otherwise light proof box, are "the eyes" of the machine. A light in front of this machine starts its driving motor, so that the apparatus moves toward the light. Any sidewise movements of the light are faithfully followed; so that the whole apparatus can be led around a room, even in a very crooked path, by merely carrying a light before it. In this "heliotropic machine" the receptors are delicately photo-sensitive selenium cells placed behind the symmetrical lenses. The effectors are (1) the electric motor, which moves the wheels of the machine just as muscles do the wings or legs of an animal, and (2) the two bilaterally symmetrical magnets, which, by changes in their relative magnetic intensity, turn the steering wheel and thus guide the machine as the relative tensions of bilaterally symmetrical muscles bend the insect body. Light increases the electrical conductivity of selenium and thus switches a current into the motor. Equal illumination of both selenium cells makes the machine move in a straight line. Unequal illumination of the two cells acts through suitable relays to control the electromagnets as motor neurones control muscles. By reversing the driving motor, the machine is made to back away from the light, thus showing "negative heliotropism."

The actual mechanism in this device is, of course, very different from that in an animal; but the principle of symmetrical arrangement, which causes movement in a straight line during symmetrical illumination and turning movements during asymmetrical illumination, is the same principle which underlies heliotropism in the living organism.

Other Tropisms.—The physiological principle of symmetry underlies the operation of all tropisms, but the mechanism differs in different cases. The tropisms, occurring in organisms other than the higher animals, involve mechanisms distinctly different from those described above. Geotropism is an asymmetrical growth response caused by the action of gravity which produces an unequal distribution of growth nutrients in a symmetrical plant. The geotropic behavior of *bryophyllum* in Loeb's experiments is shown in Fig. 96. Inasmuch as the amount of geotropic bending is proportional to the area of the leaves attached to the plant, the bending is shown to be no mere sagging but to be dependent upon the amount of the products of photosynthesis and their unequal distribution in the plant. Why this should make roots tend to grow downward (positive geotropism) and stems to grow upward (negative geotropism) in the same plant, is not explained.

Chemotropism also differs from other tropisms in certain details of its action in some organisms although the movements of fishes toward food which they "smell" (p. 420) and the movements of flies and some other insects toward the source of certain odors are probably of the same character as heliotropism, except that chemoreceptors, corresponding to the mammalian organs of taste and smell are excited rather than

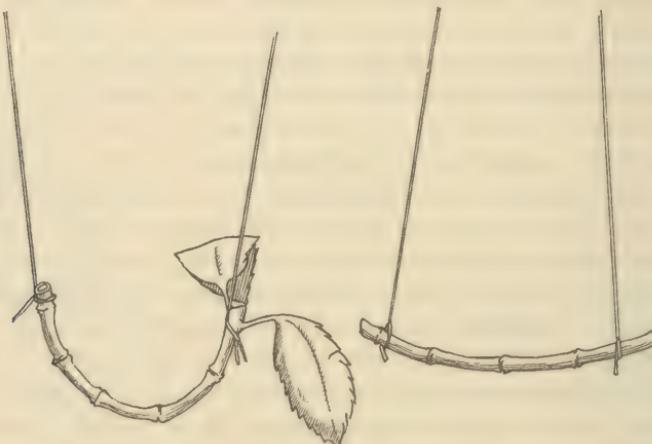


FIG. 96.—Geotropism in *bryophyllum*. The two pieces of the plant were suspended in water. After a time the piece bearing leaves has bent more than the one without leaves because the former has synthesized food to stimulate the activities of the plant. (From a photograph by Loeb.)

photoreceptors. But these phenomena have not been very successfully studied because they involve complex reactions of the animals and because a satisfactory technique for such experiments has not been devised. Much more extensive studies of chemotropism have been made, however, upon motile bacteria, protozoa and sperm cells. In these organisms, the cilia appear to be acted upon by the substance to which the organism gives a tropistic response. Owing to the symmetry of the arrangement and of the action of its cilia, the organism is forced to make certain movements in response to the chemical stimulus. Certain bacteria and protozoa, for example, move very definitely from the surrounding medium into a capillary tube from the opening of which meat juices are slowly diffusing out. Microscopic examination of their cilia shows that the movements of these organs are so governed by an unequal distribution of the diffusing substance around the ciliated cell that the organism is forced toward the source of the substance.

Instincts.—Instincts are sometimes defined as inherited habits. Accepting this definition, one would regard instincts as reflexes for which the reflex arcs are definitely laid down during the morphological development of the animal. An instinct, thus defined, is distinctly different from either an acquired or a conditioned reflex. This view has much to sup-

port it; in that an instinct, in the common meaning of this word, is an animal reaction which occurs just as effectively upon a first occasion as after many repetitions. But the development of the physiology of the central nervous system has led to a tendency to define all reactions, which depend only upon the existence of a preformed reflex arc, as mere reflexes. Instincts involve behavior which is so complex that one encounters great difficulty in attempting to explain it unless some mechanism, in addition to that of preformed reflex arcs, is assumed to play a part in such behavior. The swarming of bees at the breeding season, a typical instinctive reaction, is far too complex as regards the combination and succession of movements involved, to be called a mere reflex. Taylor has shown that part, at least, of the swarming behavior is due to positive heliotropism; for bees fail to get out of the hive at swarming time if the exit is lighted less than are other parts of the hive. The heliotropism is not as marked in bees at ordinary times as at swarming. This fact suggests that at this particular period the bee is made particularly sensitive to the controlling influence of light. This might conceivably occur as the result of internal secretion. Many insects, other than bees, show an especially marked heliotropism, either positive or negative, at certain periods in their life history. These periods are definitely related in most cases to the breeding period. It is quite possible that a combination of the action of internal secretions and tropisms causes the type of animal behavior that is called instinctive. Although much has been written on this subject, very little information based on physiological experimentation is available. The subject holds promise of interesting results from future investigation.

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CHAPTER XV

THE CORRELATING ACTION OF THE NERVOUS SYSTEM

Automatic behavior has been emphasized above as a property of tissues of lower animals. Parts of their bodies continue to function after complete separation from the rest of the organism. In many species, isolated parts can even regenerate a whole animal. Similar self-sufficiency is found in certain parts of many plants. A begonia leaf can regenerate an entire plant. But independence of separate parts is much less noticeable in the more complex organisms, especially in the higher animals. In the latter, specialization of structure, in such organs as glands and skeletal muscle, gives them a high degree of efficiency for performance of certain functions, but makes each part dependent upon the life of the organism as a whole. This dependence is especially noticeable in excitation. The more highly developed animals possess certain muscles and glands that are dependent upon the nervous system for excitation. The nervous system is chiefly dependent upon receptors for excitation. The receptors are the chief points of effective contact between a higher animal and its environment. Inasmuch as the central nervous system furnishes the conducting path between receptors and effectors, it is indispensable in enabling the organism to respond to environmental conditions. But a nervous system, even aside from its psychological functions, is much more than a mere conducting path. It adjusts behavior to the quality and intensity of stimuli. For this reason, a central nervous system is sometimes called the **adjustor mechanism** of the animal body. The nervous system is also the chief coordinating mechanism which knits together the activities of the parts of the organism and is thus the central headquarters, responsible for the unified behavior of the organism as a whole.

The Chief Divisions of the Central Nervous System.—Besides the *spinal cord*, which is the central organ for a few reflexes and a conducting path for many of them, the central nervous system of vertebrates also comprises the *brain stem*, the *cerebellum* and the *cerebrum*. The brain stem includes the medulla, continuous with the spinal cord, and is the region in which are located the roots of the cranial nerves, except those of the olfactory nerves. The cerebellum is dorsal to the brain stem and connected to it by a wide bridge. The cerebrum is the anterior division of the nervous system and varies so widely in shape and size in different animals that no general description can be given. In man, the cerebrum is proportionately much larger than in any other animal. It fills nearly

all the cranium and overlaps the cerebellum. The cerebrum rests upon the brain stem and, with the exception of the olfactory nerves, makes all its connections with the rest of the nervous system through the stem. For convenience, the chief aspects of the nervous activities which are centered in the medulla, in the cerebellum and in the cerebrum, respectively, will be discussed separately; but it is important to bear in mind that all parts of the nervous system are in organic and functional connection with one another, so that the activities of no one part are strictly independent of those of the other parts. The nervous system works as a unit organization.

Organization of the Brain.—The general distribution of white and gray matter gives a rough indication of the working organization of the brain. This is the case because white matter contains the connecting strands of nerve fibers, while gray matter contains cell bodies of neurones together with their synapses. In gray matter, then, are actual centers and "switching" devices, but white matter represents merely conducting structures. In both gray and white matter nerve structures are supported by the fibrous cells of neuroglia.

In the medulla, as in the spinal cord, both white and gray matter are present. But, although the medulla appears to casual observation to be an enlarged continuation of the spinal cord and is often called the **spinal bulb**, yet the localization of white and gray matter is distinctly different in these two structures. The spinal cord has a marked segregation of gray matter in its more central portions which form extensions toward the margins. This gives a section of the spinal cord the appearance of a letter H in gray against a white background. The spinal neurone cells are thus shown to be segregated and the spinal conducting paths to be largely located in the outer regions of the cord. In the medulla however, the arrangement of white and gray matter is much more complex, for nearly all of the medulla contains a scattering of neurone cell bodies. In numerous places, they tend to be segregated in patches easily seen with slight magnification. Some of these groups of cell bodies, arranged in bilateral pairs, have extensive connections with pairs of cranial nerves. They are called **nuclei of nerves**.

In the cerebellum and cerebrum, very important aggregations of gray matter are near the surface in the so-called **cortex**. Both these divisions of the brain are deeply and extensively furrowed or convoluted, so that the layers of neurone bodies in the cortex are much more extended than they would be if convolutions were absent. This is especially true of the human cerebral cortex which is a comparatively vast field of nerve centers. One estimate gives the number of neurones in the human cerebral cortex as over nine billions. The cerebrum also contains certain groups of gray matter in its deeper regions, in addition to that in the cortex. The white matter in all the divisions of the brain contains great

bundles of connecting fibers which form special conducting paths between the cortex and other aggregations of gray matter and between the different portions of the cortex itself. The many short branches of neurones, branches which are contained within the gray matter itself, afford innumerable connections between more or less adjacent neurones. So numerous and so intricate are the fibrous connections of the brain, that one feels inclined to the view that every neurone is either directly or indirectly in potential connection with every other neurone of the entire central nervous system. The facts of brain histology and those of nerve physiology alike tend to support this view.

The intimate details of these unnumbered connections are not all deciphered although the main features and many of the details are known. This description constitutes an important part of brain morphology. Certain of them will be described in the following sections.

The Functional Connections of the Medulla.—The medulla is a great connecting bridge. Most impulses, conducted from one of the main divisions of the central nervous system to another, must cross the medulla. It is also the region where the impulses of most of the cranial nerves must enter or leave the central nervous system. Very many of the connections between the cerebral cortex and the spinal cord cross over as they traverse the medulla so that the neurones of the cortex on one side of the brain are put in controlling connection with the spinal motor neurones which excite the muscles of the other side of the body. Nearly all impulses concerned with voluntary movements must traverse the medulla; because the cerebrum takes part in all voluntary movements and the fibers connecting it with muscles pass through the medulla, except in the case of a few of the cranial nerves. On the whole, the medulla comes nearer to being the general crossing place of all nervous conducting paths than does any other portion of the nervous system.

The Respiratory Center.—The medulla has certain unique functions in addition to those due to its many conducting paths. It is the seat of numerous **reflex centers**. The term, reflex center, refers to that part of a reflex path which is indispensable for the corresponding reflex. The center is the place where various afferent paths converge and, from thence on, use the same final common paths. But one, at least, of the centers in the medulla is "automatic," that is, independently excitable under normal conditions, irrespective of afferent impulses from receptors. This is the respiratory center. It originates impulses which are delivered by way of numerous motor neurones to the muscles which contract in breathing movements. The stimulus which locally excites this center, is some reaction dependent upon the hydrogen ion concentration of the blood. Carbonic acid is more effective in exciting this center than are other acids giving the same pH to the blood. In spite of its independent excitation, the center is markedly subject to purely reflex and to volun-

tary effects which may either excite it to a more rapid rate of discharge of impulses or may temporarily inhibit it. Yet these are only superimposed effects which merely modify the rhythmic automatic activity of the center. The breathing movements continue after the medulla is severed from most of the spinal cord or from the cerebellum or the cerebrum or even when all three of these connections are cut and the cranial nerves are also severed. So long as the paths of motor impulses from the respiratory center through the upper part of the spinal cord and the motor nerves of respiratory muscles are intact, respiration continues. The destruction of the respiratory center, as in pithing or cutting into the medulla, instantly and permanently stops breathing as does also the cutting of motor nerves to respiratory muscles. Because of its indispensability for breathing and therefore for life of the higher animals, the respiratory center is sometimes called the **vital spot**. The fatal effects of a violent blow upon the back of the head or of a sudden pressure upon the neck region, as in hanging, are due to effects upon the respiratory center. It has been located by making successive cuts across the brain stem in the upper part of the medulla until one of these cuts causes cessation of breathing. At the part of the medulla, thus located, no especial neurone cells stand out, histologically, so as to be differentiated from other structures in the same region. The particular neurones which constitute the center are therefore unknown. They appear to be arranged with bilateral symmetry, because the part of the medulla containing the center can be split lengthwise without interfering with respiration. By destruction of the tissue on one side of the center, it is shown to be so connected that each half controls respiratory muscles on its own side of the body.

The Vasoconstrictor Center.—Another center in the medulla ranks with the respiratory center in that it is indispensable to the life of a higher animal. This is the so-called vasomotor center. It is also called the vasoconstrictor center, a more descriptive term, because its excitation causes contraction of muscles in the walls of blood vessels. Its effects in constriction of arteries, especially the smaller ones, are very marked. It may also have some control over muscles in the veins, but adequate proof of this effect is lacking. This center is located by the same methods used in finding the respiratory center. The vasoconstrictor center is diffused over a larger portion of the medulla than is the respiratory center. This is shown by the fact that the cutting of the medulla at higher levels causes a partial loss of arterial constriction while further injuries in lower parts of the medulla cause increasing and finally complete loss of tone in arterial muscles. This center is also bilateral. The medulla can be split lengthwise without destroying vasoconstriction. Cutting of the spinal cord just below the medulla or cutting of sympathetic nerves severs the motor path between the vasoconstrictor center and most

of the blood vessels of the body. Such operations therefore interfere with arterial tone. Failure of the vasoconstrictor center is fatal in mammals because their arterial blood pressure is maintained largely by arterial constriction. The force pump action of the heart is not sufficient to maintain high blood pressure in arteries when they are dilated. Blood pressure may fall to one third or even to one fifth of its normal amount after destruction of the vasoconstrictor center. This low pressure does not force enough blood into the various organs of the body to maintain their life. The vasoconstrictor center is not automatic, but is maintained in continuous excitation throughout life by a constant play of sensory impulses upon it. Not only does its excitation cause vasoconstriction, but its inhibition causes vasodilatation, that is, partial loss of tone in the arterial muscles. A blush is a reflex in which the vasoconstrictor center is partially inhibited so that arteries of the skin of the face and neck dilate. Unlike the respiratory center, the vasoconstrictor center is not under even a temporary voluntary control. Its behavior is purely reflex, although the reflexes mediated by it often involve conscious states.

As explained in Chap. XI, arterial muscles are also inhibited directly in some cases. Actual excitation of vasodilator nerves, such as those innervating the salivary gland, cause relaxation of muscles in the arteries of the gland. Such nerves are not widely distributed, however, in the animal body and a nervous center for the reflexes which they mediate has not been located definitely.

Other Centers in the Medulla.—Of the numerous other centers in the medulla, the cardio-inhibitory has already been described (p. 288). The list also includes: (1) The center for chewing (mastication center), (2) the center for the sucking reflex, (3) the center for the swallowing reflex (deglutition center), (4) the center for the reflex secretion of saliva, (5) the center for the reflex secretion of gastric juice, (6) the vomiting center, (7) the center for the coughing reflex, (8) the center for the sneezing reflex, (9) the center for the reflex closing of the eyelids (winking center), and a few other centers which are located near the medulla in the upper part of the brain stem. With the exception of the chewing center, all of these mediate typically involuntary reflexes. They are not necessarily unconscious reflexes and are all affected by states of mind. The existence of centers such as these, that is, of *places to serve as the converging points of different afferent paths*, might well be presumed upon examination of a list of the many different sensory excitations which can arouse one of these reflexes. Vomiting, for example, can be aroused by the injection of certain substances into the circulation, by the presence of certain substances in the stomach, by tastes, by odors, by sights, by mechanical stimulation (tickling) of certain points of the back and roof of the pharynx, by disturbance of the sense of equilibrium

(seasickness), by recollection of previous nauseating experiences, by fear and by other emotional states. Even this is not an exhaustive list of the causes of vomiting. Similar lists can be given for the causes of other reflexes although in most cases they would not be as extended as the one above. One naturally thinks of the numerous and diverse stimuli which obviously affect the rate of breathing.

In any case, numerous different afferent paths convey impulses to each one of the centers. Most of these reflexes require consciousness in order to be effective so that they must exert an effect upon some structure in the cerebrum. The neurones which connect the cerebrum with each one of the centers of the medulla and neighboring regions are paths that are used in common by different kinds of sensory stimuli.

The extent to which centers, other than the respiratory, are automatic is not well determined. Doubtless all of them are capable of local excitation by abnormal means. For example, emetics such as the drug, apomorphine, are supposed to act directly upon the vomiting center when they are injected into the blood. But, in general and under normal conditions, these centers are purely reflex in their action.

The Autonomic System.—Several of the centers in the medulla, notably the vasoconstrictor and cardio-inhibitory centers, exert their effects through a group of nerves of a peculiar type. This large group of efferent fibers is known as the autonomic system. Autonomic refers to the degree of comparative independence, shown by the activities of this system, when largely isolated from the nervous system as a whole. Most of the purely involuntary and typically reflex activities are mediated through this system. With the exception of the control of certain muscles of the eye, none of the activities influenced by this kind of nerves are voluntary, although many of such activities are markedly but involuntarily influenced by emotional states. For example, inhibition of the secretions and movements of the digestive organs, movements of the bowels, secretion of sweat, inhibitions of the heart and vasoconstriction are involuntary reflexes, but are distinctly influenced by emotional states.

The autonomic system is sometimes called the sympathetic system, but this name is more properly restricted to only part of the extensive autonomic system. This part consists of the two chains of connected ganglia, one chain on either side of the spinal column, together with the intimate connections of these ganglia with the spinal nerves and with ganglia of the viscera (Fig. 97). The word sympathetic merely refers to the fact that these nerves correlate activities in widely separated parts of the body and has no psychological implications.

The autonomic system as a whole is characterized by affording only indirect connections between neurones of the central nervous system and effectors. Efferent fibers of neurone cells in the brain stem or the spinal cord run through cranial or spinal nerves from which they branch off to

ganglia where they form synapses with other neurones. The latter have axon fibers, usually non-medullated, connecting with muscles or glands. The fibers which connect the central nervous system with these outlying ganglia are called **pre-ganglionic**, while those connecting ganglia with

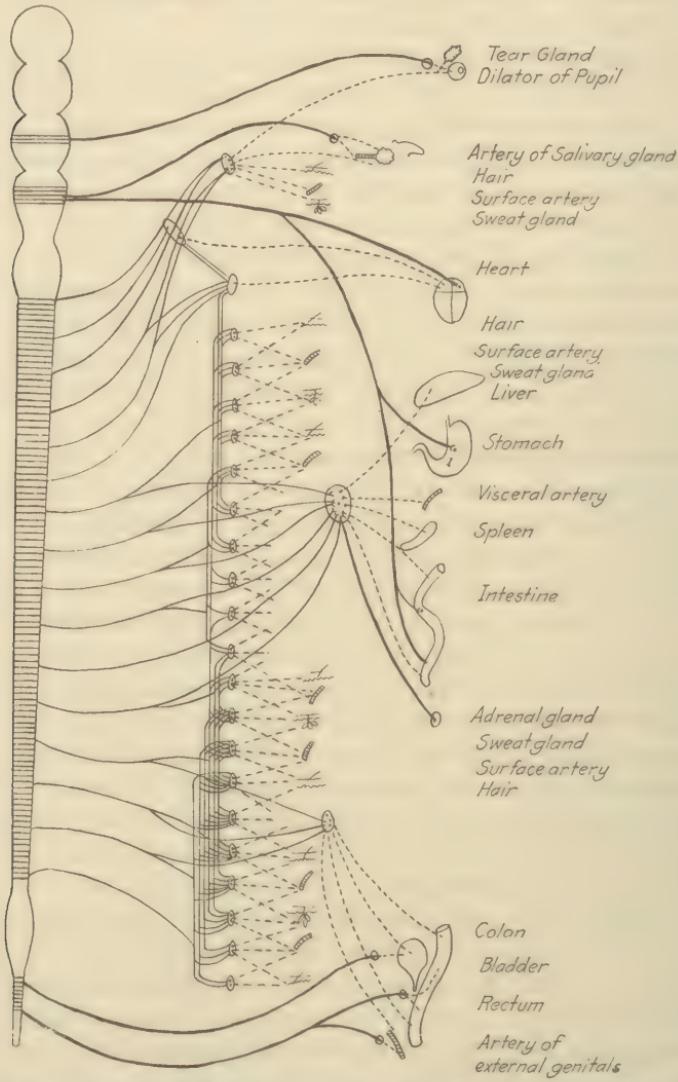


FIG. 97.—Diagram to show the principal connections of the autonomic system with the central nervous system and with various organs of the body. (After Cannon's "Bodily Changes in Fear, Hunger, Pain and Rage," D. Appleton & Co.)

effectors are **post-ganglionic** (Fig. 98). The neurones to which the post-ganglionic fibers belong are dependent upon excitation coming to them by way of the pre-ganglionic fibers.

Each efferent fiber that conducts impulses into a ganglion is supposed to make synapses with more than one of the dependent neurones. For this reason, the impulses conducted by post-ganglionic fibers are diffused into a comparatively extensive group of muscle fibers or gland cells. Thus excitation through the autonomic system is not definitely localized and does not result in accurately timed coordinated control of muscle

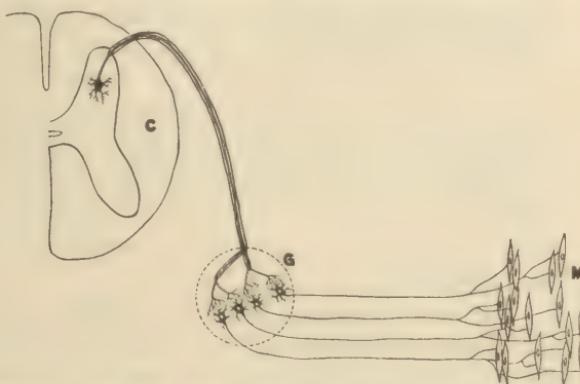


FIG. 98.—Diagram to suggest the relationships of preganglionic and post-ganglionic fibers. *C*, the spinal cord. *G*, a ganglion. *M*, muscle cells. Impulses proceeding along the pre-ganglionic fiber are diffused to a larger number of post-ganglionic ones.

fibers. But the autonomic system does not connect with voluntary muscles and the involuntary ones which it does excite, are characterized by comparatively slow and gentle contractions which maintain tonus in large groups of their fibers. For this reason, autonomic excitation is highly efficient for the type of muscular activity which it controls.

Origin and Functions of Nerves of the Autonomic System.—The pre-ganglionic fibers of the autonomic system emerge from the central nervous system at four different regions: (1) From the upper part of the brain stem, with pre-ganglionic fibers contained in the third pair of cranial nerves; (2) from the medulla with pre-ganglionic fibers contained in the seventh, ninth, tenth and eleventh pairs of cranial nerves; (3) from the thoracic parts of the spinal cord with pre-ganglionic fibers in the anterior roots of the twelve pairs of thoracic spinal nerves and those of two or more of the upper pairs of lumbar spinal nerves; (4) from the sacral region of the spinal cord with pre-ganglionic fibers in the anterior roots of the second, third and fourth pairs of sacral nerves. The origin of these four groups is indicated in Fig. 99.

The first group is sometimes called the **ocular division** of the autonomic system since it functions in the control of the muscles which constrict the pupil of the eye and those which change the curvature of the crystalline lens.

The second group is sometimes called the **bulbar division** because its fibers originate in the spinal bulb (medulla). This group functions in



FIG. 99.—Diagram to indicate the origins of the autonomic nerves in the central nervous system. 1, the ocular division. 2, the bulbar division. 3, the thoracico-lumbar or sympathetics. 4, the sacral autonomies. (After Langley.)

the control of widely scattered effectors. Among its post-ganglionic fibers are those which excite secretion in glands of the mucous membranes of the mouth and nose, in submaxillary and sublingual salivary glands, in glands of the stomach and in the pancreas. Vaso-dilator fibers (inhibitory) for blood vessels of salivary glands and of mucous membranes together with the cardio-inhibitory fibers are included in this group. Other fibers of this division excite contractions of muscles of the oesophagus, stomach and intestines and those of the bronchial tubes. The wide distribution of this group of fibers is not surprising in view of the fact that a large proportion of them are found in the vagus nerve which connects with most of the organs of the thorax and abdomen.

The third group, **thoracico-lumbar autonomic**, is still more widely distributed. Its post-ganglionic fibers for the most part join the spinal nerves and through them reach all parts of the trunk and limbs. Others extend up through the neck and have their post-ganglionic fibers distributed through cranial nerves. The fibers of this group cause vaso-constriction in the majority of the arteries of the body, erection of hairs ("goose-flesh" in man), secretion of sweat, acceleration of the heart, widening of the pupil of the eye, and inhibition of muscles of the digestive organs. So far as the last three of these effects are concerned, these autonomies are opposed to the action of certain autonomies of the first and second groups. The thoracico-lumbar autonomies are the fibers of the sympathetic system proper.

The fourth group, **sacral autonomic**, controls arterial and other nonstriated muscles of the large intestine, rectum, anus, bladder and reproductive organs. Both excitatory and inhibitory fibers are included in this group.

The first, second and fourth of these groups are sometimes put together under the name **parasympathetic**. Unfortunately, there is some confusion in nomenclature because a few writers refer to these three groups as the autonomic system. The more general convention, however, includes the entire system under the term autonomic and the thoracico-lumbar group under the term sympathetic.

One more group of nerve structures is sometimes included in the autonomic system. This is the plexus of

nerve fibers and ganglia in the walls of the digestive organs—the **plexuses of Auerbach and Meissner**. These constitute nerve networks (p. 336), analogous to the corresponding structure of lower invertebrates and represent evolutional survivals of the primitive type of nervous system. Whether they operate independently or are subject to some regulative control through fibers that do belong unquestionably to the autonomic system, is not yet known. For this reason, they are only included, provisionally, in the autonomic system under the term **enteric autonomies**.

The Sympathetic Ganglia.—The relay stations, where pre-ganglionic fibers make synapses with post-ganglionic ones, are widely distributed in the body. They are all called sympathetic ganglia, though many of

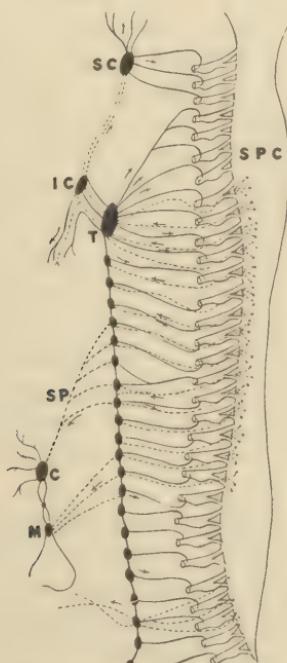


FIG. 100.—Diagram to suggest the connections between the spinal nerves and the ganglia of the autonomic system through the rami communicantes. *S P C*, spinal cord with roots of spinal nerves shown at one side. *S C*, superior cervical ganglion. *I C*, inferior cervical ganglion. *T*, first thoracic ganglion. *S P*, splanchnic nerves. *C*, celiac ganglion. *M*, inferior mesenteric ganglion. The sympathetic ganglia are shown in a chain beginning with *T*. Pre-ganglionic fibers are represented by dotted lines, post-ganglionic ones by solid lines. (After Howell's "Text Book of Physiology," W. B. Saunders Co.)

them have received special names, such as the **submaxillary ganglion**, the **superior and inferior cervical ganglia** in the neck region, the **cardiac ganglia**, the **inferior mesenteric ganglion**, and a group of ganglia with attached nerves commonly known as the **solar plexus**. But the main groups of ganglia are the two chains on either side of the spinal column in

the sympathetic system proper. These are connected to the spinal nerves by two kinds of slender nerve strands called **rami communicantes**. They are the white and the gray rami (Fig. 100). In the white rami are the pre-ganglionic fibers, in the gray are post-ganglionic ones. The post-ganglionic fibers, thus reaching the spinal nerves continue in the trunks and branches of the latter to their effector endings or to outlying ganglia through which they pass to effectors. Post-ganglionic fibers do not usually join the same spinal nerves through which the corresponding pre-ganglionic fibers emerge from the spinal cord. Indeed, the post-ganglionic fibers are generally so diffused that what might be regarded as one group, beginning in the same ganglion and having synapses with the same pre-ganglionic fibers, are dispersed through several different spinal nerves. It thus results that all spinal nerve trunks contain sympathetic fibers although not all of their ventral roots contain pre-ganglionic sympathetic fibers. Many pre-ganglionic fibers pass through one or more ganglia before making synapses with secondary neurones. Also, many post-ganglionic fibers pass through one or more ganglia in their course to effectors. Many autonomic fibers, especially those of the bulbar and sacral groups, are pre-ganglionic all the way to the organs which they innervate. In these cases, the ganglia are located in or near the several organs, so that the post-ganglionic fibers are comparatively short.

All together, the ganglia of the entire human autonomic system number over sixty. The exact number cannot be stated satisfactorily because some of these structures might be regarded as single ganglia or as separate but closely connected ones. Each ganglion can be regarded as the center of a small, subsidiary, outlying nervous system, connected with the central nervous system and serving as a relay station from which impulses coming from brain or spinal cord, are diffused.

The Rôle of the Cerebellum.—The cerebellum constitutes one of the main divisions of the central nervous system. Its neurones are of several different types. The cell bodies (gray matter) of most of them lie in the cortex. But the cerebellum has infoldings of its surface that are so deep and numerous that the cortex constitutes a large proportion of the volume of this organ.

The rôle of the cerebellum appears to be the correlation of the muscular contractions which preserve equilibrium of the body at rest or in motion. It might seem at first thought somewhat strange that an apparatus so elaborate as the cerebellum should be provided for this purpose. The cerebellar cortex contains many millions of neurones, probably more than a billion, and they are all provided with elaborate synaptic connections. Many of these connect the neurones with one another while the entire system of cerebellar neurones is extensively connected with other parts of the nervous system. Moreover, the cerebellum, in marked contrast to the cerebrum, is very highly developed in many of the so called

lower vertebrates. It is an elaborate organ in all vertebrates, but, in fishes and birds, it is especially developed in proportion to other parts of the nervous system. These animals maintain perfect poise and equilibrate skillfully in spite of rapid swimming or flying movements and in spite of powerful or fluctuating environmental pressures tending to disturb equilibrium. When the requirements for maintenance of equilibrium are understood, the usefulness of such an organ as the cerebellum is readily appreciated. A fish while swimming or a bird during flight must have nearly every skeletal muscle fiber of the entire body under a perfectly coordinated and centralized reflex control. It is not sufficient that certain muscles should respond in a coordinated reflex manner to certain sensory stimuli, but practically all muscles of the body must both contract and relax at exactly the right moment. The extent and the time of each contraction must be accurately gauged to the quality, intensity and location of innumerable sensory excitations. Even a comparatively simple set of conditions, such as those affecting the maintenance of a standing position of the human subject, requires an elaborate coordination of the reflex control of large numbers of muscles. Those which control the position of the ankle joints, the groups of flexors and extensors of the knee and hip joints, numerous muscles of the back and some other muscles of the trunk and groups of muscles of the neck and shoulders must all be brought into play. Every slight effect of gravity or of other forces, tending to disturb equilibrium, must be instantly counteracted by appropriate muscular contractions, each of which involves a nice adjustment of the relative tensions in many muscles. The cerebellum is one of the organs which aid in producing this perfection of coordination and the even more complex coordinations required for the maintenance of equilibrium during locomotion.

Effects of Removal of the Cerebellum.—Knowledge of the functions of the cerebellum is largely derived from experiments in which this organ is severed from the rest of the nervous system or is entirely removed. When it is removed from the pigeon, as in many of the pioneer experiments of Flourens, the bird, after recovering from the operation, shows a striking loss of coordinating power. It flops into unnatural positions, is unable to perch or even hold its normal position on the ground. It attempts to walk but only succeeds in staggering. Sudden movements of its head may throw the bird into a series of involuntary somersaults. If thrown into the air, it attempts to fly but falls helplessly to the ground. It is apt to make violent and uncoordinated movements even against obstacles and may thus injure itself if not protected. In time, the bird shows a considerable degree of recovery. It becomes able to perch and to walk but it never re-learns to fly very successfully and all its movements are accompanied by muscular tremors and show very imperfect coordination.

Similar experiments on dogs show corresponding results. The animal, in attempting to walk, can only stagger about. It sometimes writhes as though in pain, although other signs of pain are lacking. Its distress is probably due to terror at its loss of coordinating power. In the course of a few months, the dog shows an even better recovery from the effects than does the pigeon. The dog behaves almost like a normal animal; but at best, its muscular strength is diminished, its muscles have abnormally low tonus and it is subject to muscular tremors, especially during voluntary movements. The animal shows unusual susceptibility to fatigue.

Lesions of the cerebellum have occurred in man. The effects are somewhat similar to those described above for animals. There is a general loss of muscular tone and a tendency to a staggering gait. There is, in fact, a general deficiency of coordination. This is shown particularly in movements requiring skill of the hand. The subject may not even be able to meet thumb and forefinger without an awkward effort. Recovery from injuries to the human cerebellum are said to be much more complete than are the corresponding ones in dogs.

In general, the effects of removal or injury of the cerebellum are more permanent in the lower vertebrates than in the higher ones. This is in contrast to the effects of removal of the cerebrum. This latter operation results in comparatively slight changes in the behavior of lower vertebrates but results in very serious disturbances in mammals. Certain injuries to the cerebrum of man cause permanent paralysis in parts of the body connected with the injured portion. It seems, therefore, that with evolution of the brain, coordination and direct motor control became more and more centered in the cerebrum. The dog's comparatively successful recovery from removal of the cerebellum is thus possible because the cerebrum can, in a large measure, take over the functions of the cerebellum. In man, this process is probably still more successful.

Sensory Impulses for Equilibrium.—The cerebellum has fewer connections with spinal motor neurones than it has with the cerebral neurones and such connections as it does have with motor neurones are mostly indirect. But the cerebellum has very extensive connections and comparatively direct ones with afferent neurones. These facts indicate that the cerebellum is more concerned with the coordination of afferent impulses than with that of efferent ones. It may be likened to a great receiving station where innumerable messages are sorted, coordinated and relayed to other stations. These higher stations are the centers in the cerebrum.

Afferent impulses reach the cerebellum from several different kinds of receptors. Those especially important for the maintenance of equilibrium are: (1) Pressure receptors in the soles of the feet, (2) the receptors in the muscles, ligaments, tendons and joints, (3) the system of so-called

position and motion receptors in the semicircular canals and other structures of the inner ear and (4) the visual receptors of the eye. Not all of these are directly connected with the cerebellum. Many of them, especially groups 1 and 4, are connected with it only through internuncial neurones.

The usefulness of the first group is due to the fact that any change of position, tending to disturb equilibrium during standing, causes changes in the distribution of pressure on the soles of the feet and these changes are suitably registered in the central nervous system so as to provide for the appropriate reflex muscular activity.

The usefulness of the second group arises from the fact that any changes in muscular tension with resulting movements of joints cause mechanical stimulation of sensory mechanisms which are affected by pressures in and around muscle fibers and in the ligaments, tendons and joints. The entire motor mechanism is thus an extended sensory field from which come impulses along very numerous afferent paths for regulation of muscular contraction.

The usefulness of the third group depends upon stimulation of fine sensory endings in the linings of the semicircular canals and vestibular sacs of mammals and in corresponding organs of other animals. The semicircular canals, vestibular sacs and analogous organs are filled with a lymph-like fluid. Under the influence of gravity, this fluid exerts a varying distribution of pressure upon the linings of these organs in response to every change in position of the head. The large numbers of receptors in the semicircular canals excite the many afferent fibers in the vestibular portion of the eighth nerve. Because of these numerous connections the spatial position of the head is accurately registered in the central nervous system and calls forth intricate but perfectly coordinated reflexes which have much to do with the relative tensions (tonus) of skeletal muscles. In this way, the control of bodily postures and therefore of equilibrium is very largely dependent upon the semicircular canals. Experimental injuries to the canals have been produced by several investigators. Some of the most careful of these experiments have been done on birds. Results of such injuries are very nearly identical with the results of operative removal of the cerebellum. Corresponding to this similarity, histological evidence shows that fibers of the vestibular branch of the auditory nerve connect either directly or through one internuncial neurone with the cerebellum. The organs which in certain lower animals, correspond to the semicircular canals are called statocysts (Fig. 101). In these organs, a small concretion or grain of sand is so suspended in the fluid contents of the organ that any change in position of the animal throws the particle, by gravity, against different groups of hairlike receptors with which the cavity of the organ is lined. As these hairs are connected with the central nervous system

by large numbers of afferent nerve fibers, reflex stimulation is adjusted to the spatial position of the animal. The statocyst is thus analogous to the semicircular canals.

The usefulness of the fourth group, the visual receptors, is due to less direct effects upon the cerebellum. Probably an animal makes use of sight as an aid to equilibrium largely because of experience. This is true at any rate in the case of human beings. We have learned by experience that certain apparent motions of objects around us have a certain relationship to muscular activities that maintain our equilibrium.

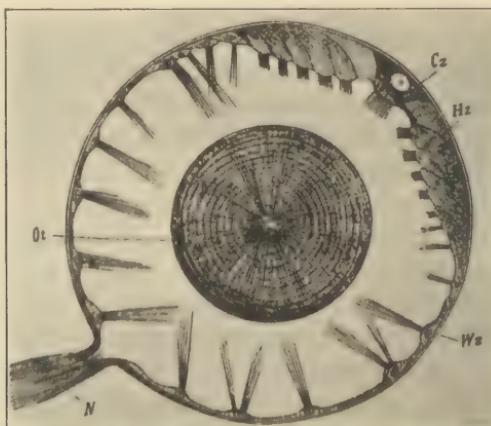


FIG. 101.—Statocyst of the mollusc, *Pterotrachea*. *Cz* and *Hz*, cells bearing fine, short hairs which are probably sensitive to pressure. *Wz*, other hair cells. *Ot*, otolith or statolith which is surrounded by liquid and is pressed against the hair cells by gravity. *N*, nerve. (After Claus.)

This is the reason why a blindfolded person finds some difficulty in maintaining equilibrium. We are also accustomed to judge distances from the apparent parallax in our visual field. This is probably the reason why many persons feel dizziness and tendency to loss of equilibrium when standing at the edge of a precipice. They miss the accustomed visual stimulus of objects in the foreground. Also, the tensions of the various eyeball muscles, as they are contracted to converge vision of the two eyes upon a given object, give afferent impulses which unconsciously influence the muscular contractions of motion and equilibrium. A combination of these visual effects and eye muscle tensions is the cause of dizziness and loss of equilibrium after whirling. During rotational movement the eyes tend to focus on specific objects in the surroundings. To do this the eyeballs have to rotate in the direction opposite to that in which the body is moving. When they can no longer focus on the selected object, the eyes are quickly moved in the reverse direction until they again direct the sight upon another object. Thus

the eyes turn rather slowly in one direction and are snapped quickly in the opposite one. This motion is called **nystagmus**. After a person has been whirled and is suddenly stopped, nystagmus continues for a time and, while it lasts, dizziness is felt and the "room appears to be going around." This is a good demonstration of the extent to which maintenance of equilibrium is dependent upon vision and the movements of the eyes. Afferent impulses from the eyes and their attached muscles are, in one sense, unnecessary for maintenance of equilibrium. Blindness does not permanently impair the poise of the body or even interfere with the performance of highly skilled acts; yet under normal circumstances the eyes are valuable supplementary aids to other receptors that are more fundamentally concerned with the control of muscular reflexes. In lower animals, as explained above, visual impulses have a direct tropistic control over muscle tensions.

Summary of Cerebellar Function.—The cerebellum apparently does exert some motor effects. Electrical stimulation of one side of the cerebellar cortex can cause movements of muscles on the same side of the body. This is in contrast to effects of one-sided cerebral stimulation, for the latter causes muscular contractions on the opposite side of the body. But the cerebellum itself has crossed connections with the cerebrum, the right side of one being connected chiefly to the left side of the other, so that experimental stimulations of the cerebellum do not prove direct connections between the cerebellum and the spinal motor neurones. But whether the cerebellum is entirely concerned with the coordination of afferent impulses or whether it functions partly in this way and partly through more direct effects upon motor neurones, its chief usefulness is the maintenance of the proper degree of tonus in the many different skeletal muscles. This extensively coordinated tonus is the cause of the posture of the body as a whole and of its parts. This tonus is also requisite for the maintenance of equilibrium and for slow changes of position. Inasmuch as all quick muscular movements are largely influenced by the degree of previously existing tonus in every muscle concerned, the cerebellum is incidentally useful in helping to perfect the coordination of muscles during locomotion and other reflex acts, in addition to maintenance of tonus.

General Functions of the Cerebrum.—The cerebrum shows a marked development in higher vertebrates so that this organ constitutes a distinctly larger proportion of the central nervous system in them than in the lower forms. This general truth is indicated by the diagrams of Fig. 102. The proportionate size of the cerebrum is especially large in animals that are capable of profiting by experience. The cerebrum has therefore come to be regarded as the organ of associative memory. This idea is well supported by physiological experiments involving removal of the cerebrum.

Decerebration is easily performed upon cold-blooded animals. Results as obtained with the frog are perhaps typical for such experiments. Removal of the frog cerebral hemispheres leaves the frog very nearly

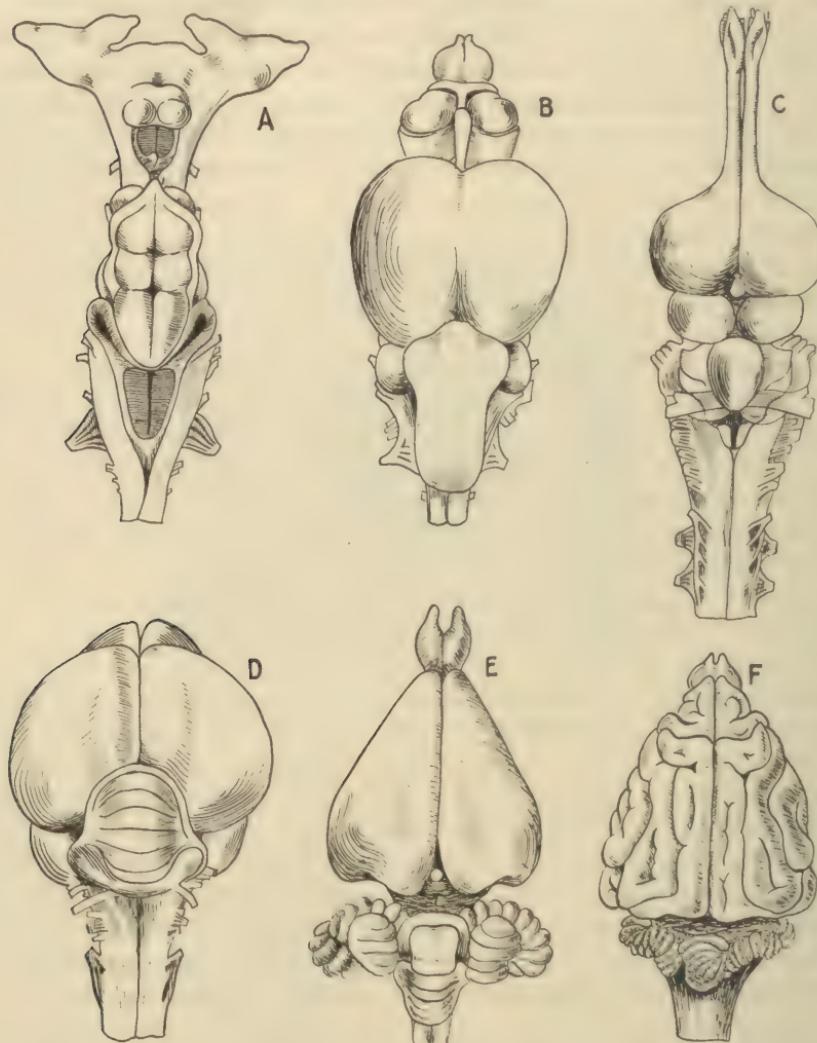


FIG. 102.—The comparative development of the cerebrum in different animals. *A*, dogfish, no true cerebral hemispheres. *B*, salmon, no true cerebral hemispheres, but a highly developed midbrain. *C*, alligator, true cerebral hemispheres, but comparatively small. *D*, pigeon, comparatively large cerebral hemispheres. *E*, rabbit, cerebral hemispheres constitute a large proportion of the brain. *F*, dog, cerebral hemispheres constitute a still larger proportion of the brain and have cortical area increased by convolutions. (After Wiedersheim.)

like a normal animal. After recovery from the operation, it maintains its equilibrium either in or out of water. It jumps and swims normally. Placed on top of a broad wheel, it moves in a well coordinated way when

the wheel revolves slowly. It maintains its horizontal position upon the top of the wheel. In short, the neuro-muscular motor mechanism of the decerebrate frog is unimpaired. The animal is, however, more machine-like and less responsive than a normal frog. Put in a pan of water of slowly rising temperature, the decerebrate frog may fail to jump out even if the temperature is raised sufficiently to kill it. Besides lack of sensitiveness, the decerebrate frog shows a lack of what might be called memory. If before the operation, it had learned the way out of a maze, it appears to have no ability after the operation to profit by that experience nor to re-learn it. The decerebrate frog does not generally jump at a threatened blow. Actual contact is usually required to stimulate it. If stroked on the back or sides it almost invariably croaks, whereas a normal frog is apt to cower and struggle when so treated. If the optic lobes and their connections are not injured by the operation, the decerebrate frog is able to "recognize" insects as food and to capture and eat them, but is so lethargic that it usually secures no food that is not actually placed in its mouth. Many inhibitions are probably removed by decerebration. Burnett describes observations upon normal and decerebrate frogs showing that in a large vivarium, the normal ones were much the more successful in capturing flies, but, when confined under a glass, they struggled to escape or crouched in fright and paid no attention to flies. Decerebrate frogs, similarly confined showed no signs of inhibiting fear but captured and ate flies put under the glass with them.

Decerebration of fishes also changes the animals so slightly that one has difficulty in detecting any differences of behavior between decerebrate and normal fishes. One significant difference can be detected. The decerebrate fish does not seek out food. This is apparently due to the failure of the sense of smell. The neurones which receive afferent impulses from smell receptors are located in the cerebrum in all animals so that receptors for this sense are no longer useful after decerebration. As fishes appear to be largely dependent upon smell receptors for the positive chemotropism which guides them toward food, they are at a loss after decerebration.

Decerebration of birds produces much more marked results. A decerebrate pigeon is very lethargic and remains quiescent, usually with eyes closed. It perches normally and has perfect coordination for both flying and walking but does not move unless actually disturbed. It pecks at the ground when hungry but does not seem to discriminate between dirt and food grains. It is thus unable to feed itself. If kept well fed it remains somnolent. Although it may waken at a loud noise, it shows no signs of alarm and quickly lapses again into an apparent sleep. It is restless and active only when hungry. The bird is comparatively mechanical and shows no signs of retaining any of the effects of its past experiences.

Decerebration of mammals is more difficult, but has been accomplished successfully with several different species, notably dogs. The decerebrate dog, as described by Goltz, is noticeably changed as compared with a normal one. After due recovery from the operation, the animal is entirely competent so far as mechanical behavior is concerned; but it lacks the qualities that one commonly refers to as a dog's intelligence. In walking, it avoids obstacles but goes around a patch of sunlight as carefully as it does around a solid object. It growls and snaps when hurt but bears no ill will toward the person who hurt it. It shows neither anger or affection nor any other signs of associative memory. The decerebrate dog, unlike a normal one, shows no signs of dreaming while asleep. Goltz describes one experiment in which a decerebrate dog was given meat and milk containing a little quinine to make the mixture bitter. The animal could not be induced to swallow it. On the contrary, a normal dog, though hesitating at the bitter taste of the same meat, swallowed it when encouraged by his master. This observation is interpreted as signifying that a decerebrate dog cannot inhibit an instinctive reaction as can a normal dog.

A general conclusion from these experiments is that the cerebrum is required for the development of reflexes such as are produced in habit forming. Presumably, all conditioned reflexes depend upon neurone associations that involve the cerebrum. Purely instinctive or tropistic reactions appear to be comparatively unimpaired in the decerebrate animal. Certain inhibitions are apparently also dependent upon the cerebrum. Associative and inhibitory functions of the cerebrum are implied in the statement that it is necessary for associative memory. This statement does not necessarily imply that associative memory is the same thing as the conscious memory which we recognize in ourselves by introspection. Physiological experiments on animals cannot deal satisfactorily with conscious memory in the psychological sense. The meaning of the term, associative memory, is shown perhaps by certain contrasts between an insect and a mammal. An ant, for example, shows a complex behavior in its social life, but its behavior is that which we call instinctive because the ant has little, if any, capacity to learn by experience. All the individuals of an ant colony behave with a nearly machine-like regularity and though one of them may have had different experiences from the others, it appears to behave like the rest of the colony. Dogs, on the other hand, have a marked individuality, which is dependent in large measure upon the stored up effects of experience. A dog may be trained to leave pet rabbits alone, but a moth probably cannot be trained to leave the flame alone. This marked difference in the power to acquire nervous reactions is parallel to the proportionate development of the cerebrum. The more the cerebrum is developed in a species, the more capable is the animal of modifying its behavior in accordance with its

individual experience. *The cerebrum is the most plastic part of the nervous system and is capable of forming the multitude of new associations between neurones, constituting associative memory in the physiological sense.*

The rôle of the human cerebrum, though including all that it does in other animals, is vastly more complex and is distinctly different in one particular. The cerebrum of man is much more important for satisfactory action of the neuromuscular mechanism than is the cerebrum of any other animal. The partial paralysis of skeletal muscles, following removal of the cerebrum of a dog, is only temporary and is almost entirely recovered from; but the paralysis resulting from certain injuries to the cerebrum of man is profound and long lasting and never shows more than a partial recovery. This probably signifies that voluntary movements are a more prominent feature of the activities of man than of those of other animals and that all typically voluntary movements involve the cerebrum. Physiologically speaking, that is, without reference to consciousness, all voluntary movements depend upon associative memory.

There are a few cases on record of children born with cerebral deficiencies. One of these lived nearly four years and upon autopsy was found by Edinger and Fischer to have only fluid in the cerebral region. Throughout its life, it showed no signs of intelligence. It never even learned to recognize its mother. Although its reflex responses were adequate for mere existence, it acquired no new reactions in addition to those possessed at birth. For example, it never learned to hold its bottle while sucking or in any way to assist in its feeding. Purely involuntary reflexes, such as those controlling digestion, appeared to be entirely normal. Sucking, crying, sneezing and many other complex reflexes were also normal. But the control of skeletal muscles in general was very defective. The child scarcely moved at all. Placed on the bed, it would lie all day in unchanged position. There is no certainty that it ever made purely voluntary motions. At two and a half years of age, it began to show muscular contracture, probably indicative of the absence of cerebral inhibition of skeletal muscles. From this time until its death from tuberculosis it was especially lethargic. In so far as this and similar but less striking cases permit of physiological deductions they indicate that the cerebrum is the organ in which occur the changes that constitute nervous and mental development of the individual.

The Localization of Cerebral Functions.—In the early part of the nineteenth century, the doctrine of phrenology was very popular. This doctrine was based on the theories of the Viennese surgeon, Gall, who taught that the cerebrum was really a group of separate organs, each having its particular function to perform. Inasmuch as the cranium is an index of the contour of the brain and each kind of mental faculty was supposed to depend upon the size of its special hypothetical brain organ, the particular intellectual capacities and the mental deficiencies of any person

were claimed to be discernable by measurements of the relative size of the different cranial prominences or "bumps." The excesses to which this theory was carried by scientific enthusiasts and charlatans, caused it to fall into disrepute. A reactionary view, that all parts of the cerebrum were equivalent, was proposed, but did not long find favor. It was counteracted by the development of the neurone doctrine, by experiments on animals and by clinical observations. The neurone doctrine clearly indicated that the nervous system was composed of functional units which, though operating in various combinations with one another, were nevertheless functionally distinct. Physiological experiments on laboratory animals showed that electrical stimulation of the exposed cerebral cortex produces contractions of muscles on the side of the body opposite to the stimulated side of the cerebrum. Stimulation at different points causes contraction of different muscles so that motor connections of the cerebrum are shown to be localized. Modern clinical observations on man have confirmed the time-honored belief that injuries to or diseases of certain parts of the cerebrum cause specific effects. Broca showed in 1861 that injuries to the left frontal part of the cerebrum were associated with loss of speech and it was later shown that the motor region for speech is entirely confined to the left side of the cerebrum in right handed persons. Paralysis of definite groups of limb muscles are also known to be associated with definitely localized brain lesions. Similarly, loss of certain sense perceptions is found to be associated with lesions in certain definite regions of the cerebrum. Blindness, partial or complete, has been produced by injuries to the occipital lobe of the cerebrum. Localization of cerebral functions, then, has been clearly demonstrated for both motor and sensory effects. Nevertheless, injuries to restricted parts of the cerebrum are not without effects upon other parts, in some cases, far reaching effects. Loss of certain sensory functions, for example, is sometimes associated in man with marked impairment of mentality. The modern view, therefore, regards the cerebrum as having its neurones in groups each of which is particularly concerned with a specific function and yet having all neurones so connected with one another that activities in any one may excite others to an extent that varies with the type of neurones concerned and with the condition of the nervous system. The cerebrum is thus a single organ and not a number of separate ones, even though each of its numerous functions is centered in a definite group or type of its neurones.

The Motor Areas.—By electrical stimulation of the exposed cortex of experimental animals and observations of muscular movements thus produced, the motor areas of the cerebrum have been mapped out. In each cerebral hemisphere, the most extensive of these areas is found just in front of the central sulcus, or *fissure of Rolando*. The most complete observations upon any one species are those made by Sherrington and his

co-workers upon the chimpanzee. Figure 103 shows the motor areas thus delimited. The entire area is somewhat larger than is represented in the diagram because it extends down into the anterior wall of the deep Rolandic fissure and, in some places, even covers the floor and a part of the posterior wall of the fissure. Other areas, also indicated in the diagram, are motor in that their electrical stimulation causes movements of

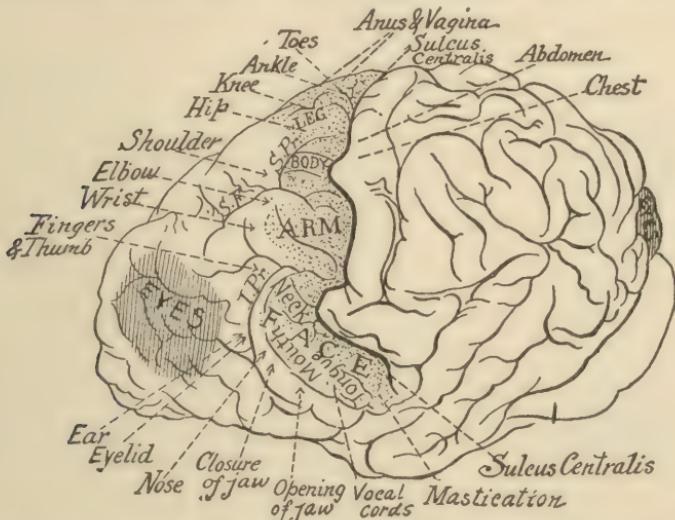


FIG. 103.—Motor areas of the cortex of the chimpanzee. (After Sherrington and Greenbaum from Howell's "Text Book of Physiology," W. B. Saunders Co.)

the eyeballs. Sherrington states that results of these latter areas differ sufficiently from effects of stimulation of the main ones to make questionable the true motor character of these areas.

The motor areas of the chimpanzee cortex are roughly analogous to the corresponding ones of other animals so far as they have been investigated. Monkeys, dogs, cats and a few other animals have been used. In man, identification of motor areas has been made chiefly by comparison between the paralysis of certain muscles and the location of injuries or tumors in the cerebrum. Cushing electrically stimulated the cortex of a conscious human patient for purposes of diagnosis. All these observations show a general similarity between the motor areas of the human cerebrum and those of the anthropoid ape.

The practical value of knowledge of these areas consists in its aid to the correct diagnosis and localization of brain injuries and brain diseases. If, for example, a bullet wound in the head causes paralysis of the right thumb, the location of the bullet in the left cerebral cortex is fairly definitely shown.

The Pyramidal Neurones.—The histological structure of the motor areas, like that of the rest of the cortex, is very complex. The many

neurones of different types show a tendency to be arranged in layers in each part of the cortex. Each layer contains a predominating number of a certain type or of a few types of neurones. One of these types has cell

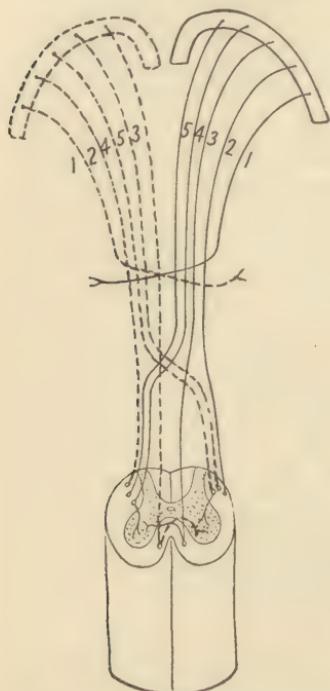


FIG. 104.—Diagram to represent the course of the fibers of the pyramidal tracts. 1, fibers to the nuclei of the cranial nerves. 2, uncrossed fibers to the lateral pyramidal group in the spinal cord. 3, fibers to the anterior pyramidal group in the spinal cord. 4, fibers that cross in the pyramidal decussation to make the lateral pyramidal tract of the opposite side. (From Howell's "Text Book of Physiology," W. B. Saunders Co.)

to have direct or nearly direct connection with motor neurones of the anterior horn of the spinal cord.

Evidence concerning the connections of pyramidal neurones, other than the giant neurones, is not very satisfactory; but it shows at least that all pyramidal neurones have numerous and far reaching connections.

Differences between the Action of Spinal and Cerebral Motor Neurones.—Injuries to the motor neurones in the anterior horns of the spinal gray matter or to any other neurones that are directly connected with effectors cause complete loss of reflex control of the muscles or

bodies that are very large compared with those of other neurones. In histological sections these cells have a roughly triangular outline and are thus shown to be shaped like pyramids. They are called the pyramidal cells and with their attached fibers constitute the pyramidal neurones. They are elaborately connected with other neurones through their numerous and many branched dendrites. Their axon fibers extend toward the center of the cerebrum. The pyramidal neurones are probably the converging points of many nervous paths. One variety of especially large pyramidal cells is found almost exclusively in the motor areas. These cells are called the **giant pyramidal cells** or the **cells of Betz**. Presumably, they form the beginning of the final common paths of the chief nerve circuits that control skeletal muscles. This idea is borne out by the connections of the axon fibers of these neurones (Fig. 104). Groups or bundles of fibers, called the **pyramidal fibers** or the **pyramidal tracts** can be traced from the cerebrum to the spinal cord through the places in the brain stem where they cross over from one side to the other. They are a part of the great group of **projection fibers**, (Fig. 106, p. 403) a term which includes all fibers connecting the cerebrum with lower parts of the nervous system. The pyramidal fibers appear

glands thus severed from the nervous system. This effect is permanent excepting in so far as neurones of which the cell bodies are still uninjured, may regenerate axon fibers. The neurones which constitute the terminal part of the final common path of any reflex, are absolutely requisite for a reflex response. On the other hand, those which form only an alternate path, a sort of shunt circuit of the reflex arc, are not requisite and, in some cases, may be destroyed without any noticeable effects upon the behavior of the animal. The pyramidal neurones are evidently parts of mere shunt circuits in most animals since they recover their motor control after decerebration. Judging from the fact that a man does not recover from cerebral injury to the same degree that animals do, one is inclined to believe that pyramidal neurones constitute an indispensable part of a particularly large number of the final common paths of the human nervous system as contrasted with other nervous systems. If this is true, it signifies that, in man, afferent neurones do not form as many direct connections with motor neurones or even indirect connections with them in the spinal cord and brain stem as they do in other animals. At any rate, the centering of nervous functions in the human cerebrum is much more marked than it is in the cerebrum of other animals.

Decerebrate Rigidity.—Some connections between sensory and motor neurones undoubtedly occur in the spinal cord and the brain stem of all animals. The behavior of the spinal animal and of the decerebrate animal gives proof of this general proposition. The phenomenon of decerebrate rigidity gives another proof. When the cerebrum of the cat, the dog or the monkey is severed from the brain stem without other injury to the neurone system, decerebrate rigidity begins as soon as anaesthesia becomes sufficiently diminished and lasts during several hours or even for a few days. Sherrington, the chief investigator of this phenomenon, has observed it to last, with some interruptions, during four days. The animal recovers in due time so that its muscles can relax normally. The muscles which go into this tonic state of contracture or spasm are chiefly the retractors of the head and neck, the elevators of the jaw and tail and the extensors of the elbow, knee, shoulder and hip. Apparently, the motor neurones of these particular muscles are normally dependent for their inhibition upon the action of cerebral neurones. Severance from the latter leaves these motor neurones open for a time to continuous excitation by afferent impulses. These impulses are shown to be the cause of decerebrate rigidity because it is a reflex phenomenon. Anaesthesia abolishes it as does cutting of the dorsal roots of spinal nerves. As Sherrington describes his observations, the cutting of afferent roots immediately makes the corresponding limb droop, as its previously rigid muscles become entirely flaccid. Cutting of the afferent roots, previous to decerebration, prevents the occurrence of rigidity. Apparently, the afferent impulses which cause rigidity originate in the

very regions of the contracture and many of the impulses probably originate in the contracted muscles themselves.

A phenomenon which appears analogous to decerebrate rigidity, occurs in human beings after injuries to the motor cortex. Skeletal muscles, in regions connected with the injured area, go into semi-spasmodic contracture. The subject is not only unable to contract these muscles voluntarily, but is also unable to relax the spontaneous rigidity of some of them. The contractures of the defective infant described by Edinger and Fischer, were probably of this type. When this condition arises in a human being, it is persistent in contrast to its brief duration in laboratory animals. It contributes to the limb deformities which are apt to follow certain brain injuries and diseases.

The Sensory Areas.—The cerebral cortex also has definite sensory areas. Their delimitation in experimental animals is somewhat difficult because of the lack of satisfactory, objective evidence of their special activities. They can be roughly located. If a certain injury to the cortex, for example, causes a complete loss of the sense of smell, this lack is detectable in the behavior of the animal and this sense is thus shown to depend upon the injured cortical region. Similarly, other sense areas are approximately located. But knowledge of these locations is chiefly dependent upon observations of the effects of brain injuries and diseases upon human sensations. Histology has also contributed largely to this subject because the connections between the different kinds of receptors and specific areas of the cerebral cortex have been extensively studied. These connections give an indication of the location of the sensory areas. None of the connections, however, are direct, but always pass through at least one relay station in the spinal cord, the brain stem, the cerebellum or the inner region of the cerebrum. This corresponds to the arrangement between motor areas and effectors, for pyramidal neurones never connect directly with muscles but only with other neurones.

At the afferent way stations, impulses from receptors are received through synapses by internuncial neurones and passed on toward the cortex. This arrangement permits some correlation and diffusion of the afferent impulses even before they reach the cerebrum. This offers an explanation of the fact that decerebrate dogs, according to observations by Goltz, respond to visual stimuli and also of the general fact that all decerebrate animals give accurate reflex responses to many and diverse sensory stimuli. These lower afferent centers or way stations have connections with the motor mechanism that permit of direct reflex responses, while the higher afferent centers in cerebral sensory areas are probably necessary for all conscious sensations and for the indirect reflex responses which we call voluntary acts.

The visual areas are located in the occipital region of the two cerebral hemispheres and the surfaces bordering the fissure between them. The

connections of this area with the eyes are indicated diagrammatically in Fig. 105. The very numerous fibers of the optic nerves receive impulses from the photo-receptors of the retinas. The fibers, to some extent, cross over in the **optic chiasma**. This decussation is so arranged in the human chiasma that fibers from the right half of each retina connect with the right side of the cerebrum and those from both left halves go to the left. The optic fibers cross over, however, to a varying extent in different species of animals and the extent of the crossing over is not known exactly even for human beings. Fibers which connect the part of the retina called the **fovea centralis** with the brain are believed to branch so as to connect *each fovea* with *both visual areas*. This is the explanation given to the fact that one-sided injuries to the occipital cortex do not destroy vision in the fovea. A person, so injured, can see with either eye, provided he so directs it that images of objects fall upon the center of the retina. If his injury is to the right occipital lobe, he is totally unable to see any object on the left of his line of direct vision, because everything in this part of his visual field would be focused upon the now useless right half of each retina. By comparison between the blanks in the visual field and the corresponding location of injuries to the occipital lobes, a scheme of the connections between the different parts of the retinas and particular regions of the visual areas has been roughly and provisionally blocked out.

The auditory areas lie upon the temporal lobes of the cerebrum. The majority of the fibers, receiving impulses from sound receptors of the ear, are supposed to connect with the opposite side of the cerebrum; but some fibers from each ear must make connections with the auditory area of the same side, because injuries to one temporal lobe apparently never completely destroy the hearing of either ear.

The olfactory areas lie upon the frontal lobe of the cerebrum, mostly upon its under surface. The synapses, which constitute part of the olfactory sensory paths are also in this general region. This fact explains the complete absence of any reflex responses to olfactory stimuli in decerebrate animals.

The taste areas have not been definitely located. Little is known about them.

The skin and body-sense areas are the locations of the endings of the afferent paths from receptors in the skin, muscles, viscera and skeleton.

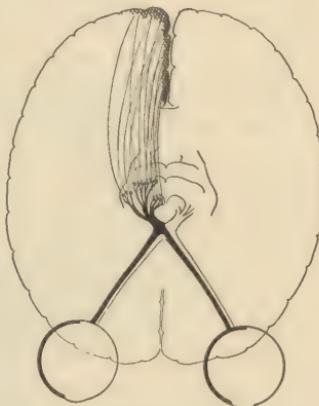


FIG. 105.—Diagram to suggest the connections between the retinas and the visual area. (From Stiles' "Human Physiology," W. B. Saunders Co.)

The areas include a large portion of the cortex, back of the fissure of Rolando, covering a broad band over the top and down the side of each cerebral hemisphere. The functions of these areas include mediation of the sense of touch, that is, pressure upon the skin, sensations of heat and cold and the sense of muscular contraction. This area was electrically stimulated in Cushing's observations upon conscious human patients. The result was a sensation of tingling or numbness in the skin suggestive of the sense of touch. No sensory response whatever resulted from stimulations in front of the fissure of Rolando.

Inasmuch as the sense of pain is largely aroused through nerves ending in the skin and the internal organs, the skin and body-sense areas might be expected to mediate the sense of pain. But evidence of any cerebral localization of pain has not been obtained. Possibly this sense is distributed through various parts of the cerebrum.

The margins of sensory, indeed, of all cerebral areas are not sharply defined but are very irregular. There is considerable variation not only in different species but even in different individuals. In some cases, these areas overlap one another. In the dog, for example, the body sense area has been shown to overlap the motor area.

Association Areas.—Connections between sensory and motor areas must exist. In some cases, fibers directly unite them. These fibers have been made out more especially in lower animals. In man, the connections are probably indirect. Large regions of the cerebral cortex are neither sensory nor motor. Electrical stimulation of such areas in the cortex of the dog or the ape produces no visible effects. It causes no motor response and does not appear to illicite any sensation. These regions are called association areas. They are shown histologically to have no direct connection with the brain stem. This means that any impulses passing through the many neurones of these areas must come from sensory areas and be transmitted to motor areas and histology shows that these regions are elaborately connected with one another and with sensory and motor areas. Bundles or strands of nerve fibers furnish these abundant connections. They are called **association fibers**. Some of them are indicated in Fig. 106, which gives an idea of the complexity of these connections. The association areas in the cerebrum of the higher vertebrates, especially the anthropoid ape, constitute a large proportion of the cerebrum. They are the great silent, unknown fields, where physiology confronts the deepest mysteries of the brain, the most complex mechanism known to man.

As higher animals have a greater development of these areas and are also especially capable of acquiring habits and conditioned reflexes, the natural inference is that the association areas are the plastic parts of the nervous system which develop and change so as to register the effects of individual experience. Neurones of association areas are known to

develop later than do those of other parts of the nervous system. These great areas are probably blanks at the time of birth and upon them is recorded the story of a lifetime. The mechanism for making these

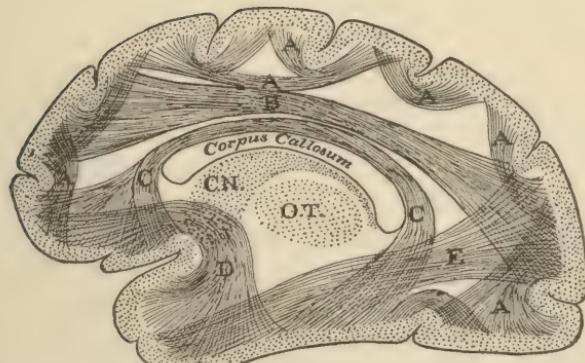
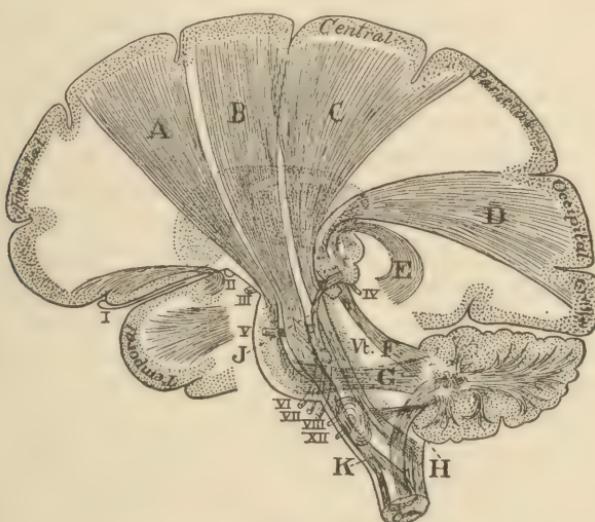


FIG. 106.—Projection fibers (upper figure) and association fibers (lower figure) of the brain. Projection fibers. A, connecting frontal areas with nuclei of the brain stem; B, motor (pyramidal) tracts. C, sensory tracts. D, visual tracts. E, auditory tracts. F, fibers between the cerebrum and the cerebellum. G, fibers between the cerebellum and the brain stem. H, fibers between the cerebellum and the cord. I, fibers between adjacent parts of the cerebrum. J, a short tract between the auditory nucleus and the brain stem. K, decussation of the pyramidal tracts; Roman numerals refer to the origins of the cranial nerves.

Association fibers. A, between adjacent or neighboring association areas. B, between frontal and occipital areas. C and D, between frontal and temporal areas. CN, caudate nucleus. OT, thalamus (these last two contain gray matter); Corpus callosum, the largest of the groups of association fibers which connect the cortex of the left cerebral hemisphere with that of the right. (After Starr, from Howell's "Text Book of Physiology," W. B. Saunders Co.)

records is suggested by the phenomena of conditioned reflexes. These point to the formation of neurone connections such that different afferent

paths become associated with the same final common path. This would account for the way in which the sound of a bell can be made to take the place of the sight of food as a stimulus for secretion of saliva.

The nature of the changes, which produce these connections, is unknown; but considerable research has been directed toward this problem. The investigations of Ebbinghaus on the rate of learning, that is, the rate of conscious acquisition of memory impressions, and also on the rate of forgetting, the fading of memory impressions, together with many similar investigations, point to an actual chemical change as underlying the process of memory. Learning is shown by these investigations to proceed according to the same time laws that govern certain types of chemical reactions. Conscious memory is not unrelated, physiologically, to conditioned reflexes; for the latter appear to be nothing less than the motor expression of memory. It is thus highly probable that chemical alterations of components of neurones are made during the acquisition of new cerebral connections. This conclusion does not in any way preclude the possibility of some change in the physical properties of the neurone, presumably at synapses, as the immediate cause of the establishment of new connections. But it does emphasize the probability that a mere physical change, comparable to the opening and closing of a switch, is inadequate to account for the formation of new synaptic connections.

The association areas are supposed to be the parts of the brain that are requisite for what we call intelligence. This idea is based chiefly upon the observation that serious injuries to *motor* and *sensory* areas have occurred in human beings without any apparent loss of mental powers. In spite of blindness, deafness or some other cerebral anaesthesia, or a partial paralysis, such individuals appear to reason as clearly as ever. On the other hand, injuries to association areas have been observed to result in impairment of intelligence or in insanity. None of these observations are sufficiently controlled to furnish adequate proof; but the general impression is that the association areas are the seat of memory and of intelligence. If this is true, the obvious differences in the native mental capacity of human individuals must be determined by the inherited organization of the neurone structures of these large association areas; while all that education and other formative influences do to change an individual, must be effective through further organization of these same structures.

The Grouping of Cerebral Functions.—Even a cursory study of the association and projection fibers of the cerebrum shows the complexity of the connections between cerebral neurones and thus suggests a picture of the interrelationships of their functions. Any attempt at an exhaustive description of even those functional connections that are known from histological evidence, would require an extended treatise in neurology;

while descriptions, based upon psychological evidence, would be still more extended. A brief description of one grouping of cerebral functions will serve as an example. This is the grouping that has to do with language.

The Language Functions.—The sensory functions, chiefly concerned with language, are hearing and sight, though others may be involved. The child learns to associate certain sounds with a definite concept and soon distinguishes specific spoken words as having a meaning. Words with short repeated syllables as ma-ma and bye-bye take on a significance which is expressed in appropriate action at a very early age. Later, the child comes to recognize printed syllables and soon word concepts become attached to them. But the blind person can also acquire the same concepts through the sense of touch and even the sense of smell may convey the same ideas that spoken or written words would carry. The language of flowers is not only a poetic figure but refers to the psychological fact that many individuals experience vivid impressions owing to associations with certain odors. In some measure, any sensation may become so associated as to be the stimulus for a language concept. The significance of words thus becomes definite and normal in the mind of a congenitally blind person after suitable training. Slowly, but at last successfully, the sense of touch, aided by the muscle sense and by taste and smell, accomplishes changes in the brain, which would be dependent upon sight and hearing in normal persons.

The motor functions, concerned with language, are chiefly the acquired, coordinated reflex responses of the muscles used in speaking and of those used in writing; but other acts may also be involved. Deaf mutes are not the only people who "talk with their hands." The trained soldier on drill makes accurately adjusted motor responses to words of command with automaton precision that can be only reflex. Probably the coordinated action of any group of muscles can become associated with language concepts.

Motor Aphasia.—The effects of injuries to the human brain have shown the distinctions between these different sensory and motor functions, disentangling them, in a figurative sense, from their acquired associations. Certain injuries cause the loss of the power to speak words. This is the condition called motor aphasia. The person so afflicted appears to understand words and to correlate them with other mental conceptions. He can even write them, but is unable to coordinate for word utterance. Various degrees of this difficulty have been observed. Broca described a case in which the individual could use no word for numbers except three and was obliged to make this do for all numbers. Cases have been recorded where certain nouns could not be uttered while other words came as usual. Aphasia sometimes involves complete loss of the power of speech. In some cases, words can not be spoken but can be sung.

The cause of aphasia is presumably injury to the so-called **speech center**. As explained above, many injuries or lesions in this region have been found upon autopsy of persons who have had aphasia. This center is not, however, a motor area in direct connection with muscles of speech. The center is rather a part of an association area and is the cerebral region where "memory" makes connections with the specific motor neurones that control these muscles.

Agraphia.—Another similar difficulty is the failure of the ability to write. This condition is called agraphia. The person so afflicted is able to talk, but has lost the power to associate word ideas with the muscular coordinations necessary for writing, although he had previously acquired facility in it. He is in much the same condition as an illiterate person who speaks his own language but cannot write it, only the illiterate has never learned reading and writing, while the agraphic person may have learned both but has later lost his writing ability.

The cause of agraphia is assumed to be an injury to a portion of the cerebral cortex in the association area near the motor centers which control muscles of the hand and arm. It is called the **writing center**.

Sensory Aphasias.—Other language difficulties, distinctly different from motor aphasia and agraphia, are called **sensory aphasias** or **amnesias**. Two kinds are distinguished: Inability to understand spoken words, word-deafness, and inability to understand written words, word-blindness. In cases of word-deafness, the patient may be likened to a person listening to the chatter of foreign tongues, for he appears to know that words are being spoken but they have no meaning for him. In cases of word-blindness, the patient may be likened to a person gazing at unknown Egyptian hieroglyphics, for he appears to know that the printed page should convey meaning but he cannot understand it.

The Hypothetical Language Centers.—Figure 107 shows approximately the supposed location of the four centers which are assumed to mediate the associations, the failure of which results respectively in motor aphasia, agraphia, word-deafness and word-blindness. Lack of definite knowledge about these centers is due to the difficulties involved in their investigation. In the first place, the problem cannot be approached by experimental method because only human beings can serve as material. The only method available is observation of the effects of cerebral injuries and brain diseases. Accurate results from this method require the cooperation of the competent psychologist, observing behavior and mental life of the subject, and of the trained neuro-pathologist, recording the histology of the brain after autopsy of the same subject. In the second place, the anatomy of the cerebrum varies widely in different individuals. Sherrington found the convolutions of forty chimpanzee cerebrums to be so varied that he was unable to use a drawing of the brain of any one of them as a chart upon which he could plot the

locations of motor areas of the others. Probably the variations of the human cerebrum, as regards the locations of specific areas, are even more marked. In the third place, injuries to and diseases of the brain are not apt to be strictly localized but generally involve various regions and thus so complicate the resulting abnormalities of mind and action that correlation between the nature of the injury and the character of its effects is very hazardous.

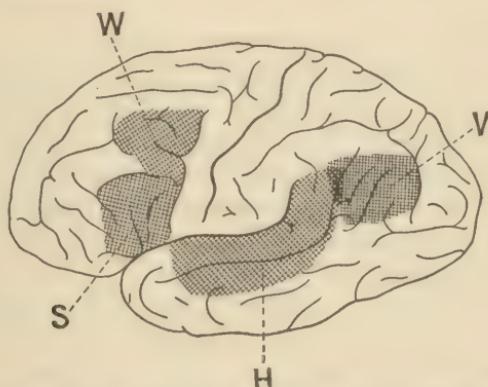


FIG. 107.—The supposed location of the language centers of the human cortex. *W*, area in which injuries are supposed to cause the loss of the power to write words (agraphia). *S*, area in which injuries are supposed to cause loss of power to speak words (aphasia). *V*, area in which injuries are supposed to cause loss of ability to recognize printed words (word blindness). *H*, area in which injuries are supposed to cause loss of ability to recognize spoken words (word deafness). (After Donaldson, from Howell's "Text Book of Physiology," W. B. Saunders Co.)

The Unifying Interrelations of Cerebral Functions.—Aphasia and amnesia are not the only associative failures that have been observed. Probably any kind of a particular *motor coordination*, which is a reflex response to conscious sensory impressions, may be lost as a result of brain injuries or diseases. The general term **apraxia** is used to cover all such motor failures. An example is the loss of ability to use certain tools. Also any kind of a *sensory impression* may fail to arouse a response that could be called intelligent. The general term **agnosia** is used to cover all such sensory failures. An example is the loss of ability to recognize musical sounds—*amusia*.

It should be clearly understood that an apraxia is distinctly different from a paralysis. The muscles involved in any apraxia, are still under voluntary control even for highly coordinated movements; but they cannot be coordinated for responses to certain particular combinations of sensory impressions. A person with aphasia, for example, can utter sounds that require complex coordination of the muscles of speech; but he can not utter the sounds that should come in response to word ideas, even though his intelligence conceives the words with normal clarity, strives to express them in speech and does successfully express them in

writing. Voluntary *motor* centers are not necessarily involved in an apraxia. The really characteristic cause of this difficulty is a failure in a specific association center.

It should also be clearly understood that an agnosia is distinctly different from a cerebral anaesthesia. The receptors involved in any particular agnosia are still useful to the afflicted individual and may even convey to his intelligence highly complex associated impressions; only certain particular impressions fail to become coordinated in his intelligence. A person suffering from word-deafness, for example, hears all sounds and appears to know that he hears them, but the sounds of words fail to awaken any meaning in his mind, even though the sounds of music awaken his normal appreciation and pleasure. Specific *sensory* areas in the cortex are not necessarily involved in an agnosia. The really characteristic cause of this difficulty is apparently a failure in certain centers which so synthesize specific sensory impulses that they produce a coordinated, useful mental impression.

In apraxia and in agnosia, certain "cogs" in the coordinating mechanism are put out of gear while the remainder of the brain organization continues to function, perhaps, in a normal manner. But while these abnormalities show that complex, reflex acts may occur with a certain degree of independence, yet the very complexity of the associations, which these abnormalities reveal, emphasizes the close knit interdependence of the brain neurones. The development of the mental life of Helen Keller or of other less famous blind deaf-mutes is striking evidence to show that one group of cerebral sensory neurones may act vicariously for other groups. This could be possible only in a closely interconnected neurone system. It would not be possible if association areas were not functional. Presumably, the association neurones are the warp which holds the intricate pattern of the billions of cerebral neurones in a unified whole.

The extent to which one association area may itself act vicariously for another may only be surmised. Recovery from motor aphasia has been claimed to occur in young persons, though this difficulty seems to be more permanent when it afflicts an adult. A tentative explanation of recovery from this and other apraxias is suggested by the idea that associating centers other than the usual ones may form certain connections between sensory and motor areas and thus assume the functions of the injured centers.

Leaving consciousness out of account, the elaborate coordination through association areas is not essentially different from the correlating action of other parts of the human central nervous system and of the synaptic nervous systems of lower animals. But in effect, the associative powers of the human cerebrum are very different from those of any other nervous structure because they are so vastly more complex and so much

more plastic and adaptable. They constitute the faculty which has given to man the power to react diversely to his environment and to dominate it to a greater degree than does any other organism. The associative powers are also the most striking of those forces which tend to integrate the infinitesimal parts of the material body and the fragments of the mental life into one functional whole.

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CHAPTER XVI

RECEPTORS

The ability to respond to environmental changes is necessary in animals in order that they may obtain food and escape from danger. Receptors afford contact between the animal and its environment so that effective reflex responses result from excitation. Nerve fibers themselves are not easily excited but receptors are especially adapted to excitation by minute changes and can thus start nerve impulses. The contrast between the excitability of nerve fibers and receptors is shown by a comparison between the pressures required to stimulate exposed nerves and those that stimulate the skin of a frog. The nerve requires considerable pressure while the frog jumps at the slightest touch. Moreover, each type of receptor is adapted to respond to minute stimuli of a particular kind as, for example, light, heat, pressure or sound and inasmuch as each receptor has special connections with effectors through the central nervous system, this specialization of receptors tends to insure the appropriate response to a given stimulus. The usefulness of receptors is also made apparent by the observation of the sensitiveness of each type of receptor to its particular kind of stimulus. Sound receptors of the ear are sensitive to minute air pressures which do not affect any other receptors, even the pressure receptors of the skin. Smell receptors are excited by minute concentrations of odorous substances which cannot excite any other receptors. This specialization is found in every kind of receptor. Each one shows a threshold stimulus that is lower than the threshold for the same kind of stimulation in any other type of structure. A receptor thus has selective excitability. Receptors are sometimes described as mechanisms for lowering the threshold value of specific stimuli.

Receptors Not Necessarily Sense Organs.—The physiology of receptors is apt to be confused with the physiology and psychology of the senses. One naturally thinks of the eye as the organ of sight rather than as a photo-receptor, of the ear as the organ of hearing rather than as a sound and position receptor. The study of the senses has received a great deal of attention because of its obvious practical application to human physiology, to medicine and to psychiatry. But from the standpoint of general physiology, the study of conscious sensation as a measure of the activity of receptor organs, has marked limitations. The receptors of lower animals or even plants must be investigated and some receptors

do not affect conscious sensation, even in man, while some sensations take place, as in hallucinations and dreams, without the excitation of receptors. In view of these limitations, modern physiology has followed the suggestion, proposed by Bethe (1897), that the term, receptors, be used in place of the older one, sense organs. The activities of receptors, in experimental animals, can be detected and measured without any reference to conscious sensation. This is due chiefly to the phenomena of reflex action. The reflex response is a sign and a measure of receptor excitation. In spinal and decerebrate animals, excitation and its response are presumably devoid of conscious sensation. The phenomena of tropisms give even more useful information concerning receptor excitation. All tropistic responses are capable of interpretation without any reference to consciousness. The electrical variations of afferent nerves also serve as indicators of excitation of the corresponding receptors. It is true that investigations of receptor activities in man and the higher animals have been greatly assisted by sense physiology. For example, nearly all that is known about the excitation of eye receptors by different wave lengths of light is derived from observations dependent upon the human sense of color vision. Most of our knowledge about skin pressure receptors is derived from similar dependence upon the human sense of touch. In the main, however, it is an advantage to recognize the fact that sensation is only an incidental result of receptor excitation. Sensation is dependent upon the cerebral cortex, which in the higher animals is a superstructure of elaborate shunt circuits added to the central nervous system. So far as functional usefulness of receptors is concerned, consciousness is not strictly necessary although the effectiveness of motor response is, in some way, greatly increased by the senses. This increase is especially noticeable in human beings for in them, light receptors and sound receptors are so connected through the cerebrum that they are perhaps useless, except when able to arouse conscious sensation.

Müller's Law of the Nature of the Quality of Sensation.—The specific effect of the excitation of a given nerve is due, as explained above, to the specific response in the particular structure in which the nerve fiber ends. This idea was originally based, in part, upon the physiology of the senses and was first formulated by Müller. It is commonly known as Müller's doctrine of specific nerve energies. This term is misleading as it seems to imply that the energy in different nerves is specific to each one, whereas it is really used to refer to the very opposite of this. Müller recognized that different kinds of stimuli, applied to the same receptors or to their nerves, give the same quality of sensation. Mechanical pressure on the retina causes a sensation of light. Electrical stimuli, administered so as to excite the retina, give a sensation of light. The character of the stimulus does not determine the general character of the sensation. Cutting of the optic nerve is said to give a sensation as

of blinding light. Mechanical stimulation of the ulnar nerve or of other nerves containing afferent fibers from the skin, gives a sensation of skin pain and touch. Thus, excitation of the receptor itself is shown to be unnecessary for securing sensation of a given quality. Yet sensations are themselves highly specific. Introspection shows that the sense of sight is distinctly different from the sense of hearing, notwithstanding the phraseology of modern art which discusses the tones of color and the color of tones. Yet one knows that these senses are not comparable except in an imaginary way. A given light cannot be judged to be brighter or darker than a given tone is loud or soft. Each sensation has what is called its own *modality*. These and other similar facts lead to the modern form of Müller's doctrine which assumes that the character of a sensation is determined solely by the particular part of the cerebral cortex to which the sensory impulse is conducted. DuBois-Reymond's famous statement to the effect that, if the eye were connected to the auditory centers and the ear to the visual centers, we should hear the lightening and see the thunder, epitomizes the Müllerian doctrine. It is part of the larger truth that specificity of the effects of any nerve excitation is determined by the place or mode of ending of the nerve.

The most satisfactory proof of this proposition is given by observations of the electrical variations of nerves. In any nerve trunk, this variation has the same general character as in any other, differing only in accordance with the number of fibers excited and the rate of their excitation. Nerve impulses are thus all alike qualitatively and differ from one another only in magnitude, velocity and frequency. Along a given stretch of one nerve fiber of uniform structure, composition and temperature, the impulse does not vary in magnitude ("all or nothing" principle) nor in velocity and the only differences that can occur are in the relative frequencies with which the separate impulses follow one another. Neither the nature nor the intensity of the exciting force has any effect upon the individual nerve impulses nor does the location of the excitation, whether in a receptor or upon a nerve trunk, alter the character of the resulting nerve impulse.

The uniform quality of all nerve impulses does not seem peculiar or startling when one remembers that all forms of energy can be converted into an electric current if the right kind of energy transformer is used. Heat is converted by the thermopile, mechanical energy by the dynamo, sound by the telephone, energy of chemical reactions by the galvanic battery, light by photosensitive chemical reactions. Just as these mechanisms can produce an electric current for conduction along a wire, so the different types of receptors, although responding to different forms of stimuli, can produce nerve impulses for propagation along neurones.

The Weber-Fechner Quantitative Law of Sense Excitation.—The effects of different intensities of stimulation of receptors are recognized in conscious sensation. Introspection shows that we detect differences in the intensity of lights, loudness of sounds, amount of skin pressures and intensities of smells and tastes. Weber found that if a certain difference between the intensities of two stimuli was just enough to be appreciated as a difference, then the difference between two other intensities of the same stimulus must be proportionately great to be similarly appreciated. For example, suppose that with a weight of 30 gm. upon the hand, the addition of 1 gm. is just sufficient to give the sense of greater weight, then with 60 gm. upon the hand, a proportional increase of $\frac{1}{30}$, or 2 gm., would be required to be felt as an additional weight. Fechner formulated this law mathematically. He showed that the intensity of a sensation is proportional to the logarithm of the number which represents the comparative strength of the stimulus. This law is generally known as the Weber-Fechner law. It has limitations in that it holds true only for stimuli of a certain medium range and does not apply to stimuli of comparatively low or high intensity.

Excitation by Nocuous Stimuli.—Sherrington proposed the term, nocuous stimuli, for those which tend to cause or at least foreshadow injuries to the organism. Responses to such stimuli are peculiar and put this kind of excitation in a class by itself. It is therefore conveniently described in advance of other receptor excitations. The word nocuous is interchangeable with painful, as applied to stimuli that act upon conscious beings. But painful implies a psychical reaction and thus makes unwarranted assumptions with reference to most animal species. Nocuous stimuli have one feature common to their action upon all animals with a nervous system, namely, the tendency to produce a reflex response which affords protection or escape from the threatening stimulus. This response can be investigated by strictly physiological methods. As it usually occurs in decerebrate animals, the actual sense of pain is shown to be added on to the simpler reflex response, so as to afford additional insurance of escape from danger.

The role of protective responses in the preservation of the species is so obvious that one is not surprised to find that **nociceptive nerves**, those so located as to conduct impulses from nocuous stimuli, are present in very primitive animals as well as in higher ones. It is also to be expected, as is the fact, that such nerves would be widely and abundantly distributed in the body and especially in the surface integument which comes into actual contact with destructive forces of the environment. Correspondingly, we find "**pain spots**" in the human skin so distributed that only the finest needles can be used to find a spot at which a prick does not elicit pain. Nevertheless, pain can be produced by skin injuries only through excitation of one or more of these spots.

Histologically, no definite receptor organs can be found at such points. Finely branched naked nerve endings occur there in deeper layers of the skin. These endings are apparently the nociceptive mechanism. So far as is known, naked nerves are the only structures that are provided in any animal for an excitation that causes protective response to typical nocuous stimuli. Such nerve endings are called **nociceptors**, by Sherrington, even though they are not definitely specialized receptors. They serve their special function particularly well, since, as shown above, nerve fibers are not as easily excited as are typical receptors. It would probably not be an advantage to an animal to be excited to flight or other protective response by forces too weak to threaten real danger. Moreover, a non-specialized nociceptive mechanism tends to insure response to all types of injurious forces. We know, indeed, that the sense of pain is excited by any sufficiently strong cutaneous stimulation, whether it be chemical, as by mustard, strong acid, etc.; or mechanical, as by cutting, pinching, etc.; or electrical, as in application of strong induction currents; or thermal, as in burning or freezing; or osmotic, as in action of water or salt in a cut.

It is nevertheless true that some stimuli are more adequate for excitation of certain nociceptive mechanisms than are others. Pain is not caused, apparently, by cutting the gall duct of a dog and surgeons state that in human beings no signs of reflex effects are aroused by cutting of that region; yet excruciating pain is caused by the passage of a gall stone through the duct. This indicates that excitation occurs only in response to an adequate stimulus. Distension of the gall duct by injection of an innocuous liquid, physiological saline, gives reflex effects in an anaesthetized dog. These effects include fluctuations of blood pressure that are equivalent to pain reflex responses.

Nocuous stimuli arouse special nerves. This is shown by the *punctiform distribution* of skin spots at which the human sense of pain can be aroused. Stimulation of one of these points gives pain if it gives any sensation at all. Stimulation of other points of the skin may cause sensations of touch, of heat, or of cold, but does not give the sense of pain, no matter what the character of the stimulus may be—provided, of course, that the stimulus can be so restricted that it does not spread to any nearby “pain spots.”

Further and more convincing evidence of the existence of special nociceptive mechanisms is shown by the specific character of the protective responses and the imperative way in which they occur. They are definite reflexes and they cannot be inhibited by circumstances which might shut off others. Sherrington classes the protective responses with what he calls **prepotent reflexes**. By the latter, he means those which take precedence over other reflexes. When several afferent arcs are excited, simultaneously, they may compete, as it were, for use of the same

final common path. For example, stimulation of a pain spot in the skin of the foot and of a pressure receptor in the same region would start afferent impulses along two different kinds of nerves. The response to each of these would include contractions of leg muscles, but the motions would be differently coordinated in the two cases. Sherrington found that a needle prick of the foot of a decerebrate dog excites the response which he calls the *flexion reflex*. In this, the limb is drawn up as it would be by a conscious dog with a wounded foot; while the other legs are slightly stiffened for standing on three legs. But a touch or mild electrical stimulation of the foot excites the response which Sherrington calls the *extensor thrust reflex*. This is similar to the initial motions of walking. These two different stimulations, then, excite distinctly different responses. But if the pricking (nocuous stimulus) is given, the resulting flexion reflex is absolutely prepotent over the extensor thrust. The latter cannot be produced so long as the flexion reflex is being excited. This is only one of numerous cases in which the responses to nocuous stimuli are shown to be prepotent.

Further evidence is found in the reactions of conscious human beings. The sense of pain tends to be prepotent over other sensations. A person in intense pain can concentrate upon reading or listening only by a great effort. It is also noticeable that reflex responses to painful stimuli are involuntary and can be inhibited only in the individual of rare self control. One jumps or at least twinges when burned or wounded and may do so even if previously warned and exerting the will to inhibit the motion.

As evidence of the way in which reflexes are adapted to the preservation of the species, the prepotency of reflexes having to do with reproduction, is remarkable. Reproduction takes precedence over the preservation of the individual and correspondingly, even nocuous stimuli are not always prepotent over sexual ones. An illustration of this is seen in the behavior of the male frog at breeding season. It shows the clasping reflex in which sustained contractions of muscles, especially developed at this season, clasp the female with the fore limbs of the male. This response is a simple, spinal reflex for it occurs in the pithed male frog at breeding season. The stimulus is a touch of the skin of the inside of the fore limbs or of the sternal region. When so produced, in the spinal frog, clasping is not interrupted by cutting, burning or other nocuous stimuli administered to the animal. So long as it has anything to clasp its fore limb muscles are not used for other responses. In other words, the clasping reflex takes possession of the final common path and is prepotent even over nocuous stimuli.

Classification.—There are probably more than twenty different kinds of receptors in the human body. The exact number cannot be stated because opinions differ as to whether certain of them are sufficiently distinct to be called a separate type. For example, there appear to be

four distinct kinds of receptors called taste buds, but there may be other kinds. The number of different kinds of receptors for smell is very difficult to determine and the grouping of other receptors is still more hazardous. Indeed, all attempts at a complete classification of receptors meet with difficulties. These are pointed out below. Nevertheless, many receptors show such likenesses and differences in their respective activities as to make the comparison and grouping of such organs an aid to their study.

Classification of the Senses.—The old classification of the senses recognized two groups; the special senses and the general ones. The special senses included those dependent upon specific end organs. Sight, hearing, smell, taste and touch, the five special sense, were thus put in a class by themselves. The general senses, such as hunger, thirst, fatigue, etc. not apparently dependent upon specific receptor organs, constituted the other class. This classification is not applicable to all animals for in some the photo-receptors are scattered over the body instead of being localized in eyes. The receptors for olfactory stimuli are also widely distributed in some animals, being present in all parts of the skin of many invertebrates.

Another classification of the senses is based upon introspective psychology, in that it groups sensations according to the nature of the psychic effects which they produce in human beings. This classification recognizes two main groups. The first group includes the external senses, hearing, sight, smell, pressure on the skin and sensations of heat and cold on the skin. These are called external senses because one mentally projects the stimulus to some source outside of the body. The other group includes the internal senses: Pain, muscle sense, hunger, appetite, thirst, sexual sense and possibly others. These are called internal senses because the stimulus is felt to be something within the body. The tendency to distinguish between external and internal senses is reflected in our every day speech. We say that we see *objects* and hear *sounds* but that we are thirsty. Classification upon this basis is not entirely satisfactory because our mental projections are not always the same. Heat and cold are sometimes felt to be external, so that we say "it is hot" or "it is cold;" but they are sometimes felt to be internal so that one says, "I am hot" or "I am cold." The sense of touch is similarly confused.

Classification of Receptors.—Sherrington has proposed a classification of receptors: (1) The extero-ceptors, stimulated by forces outside of the body; (2) the proprio-ceptors, stimulated by activities that occur within the body itself and (3) the intero-ceptors, stimulated by substances or conditions within the digestive cavities but not in the body itself.

Parker has proposed a useful classification based on the kind of force exciting the receptor. This classification recognizes: (1) The chemo-receptors such as those of smell and taste which are excited by suitable

concentrations of substances in solution; (2) the mechano-receptors, such as those for sound, for pressure on the skin (touch) and for effects of muscular contraction, receptors which are excited by mechanical pressures; and (3) radio-receptors, such as those for light, heat and cold, which are excited by radiant energy. This classification encounters a difficulty in placing the receptors for the sense of pain for these are all excited by widely differing forces. Another difficulty is the fact that many receptors can, under some circumstances, be excited by stimuli other than their normal ones. But this classification is useful in that it suggests the different kinds of physiological processes occurring during normal excitation in each of the different kinds of receptors.

Some receptors cannot be satisfactorily classified because they have not been identified anatomically. Receptors for the senses of hunger, appetite, thirst and certain internal pressures have never been identified. In some of these cases, the physico-chemical character of the adequate stimulus can only be conjectured. So far as these limitations permit, receptors are grouped according to each of these schemes as follows:

CLASSIFICATION ACCORDING TO SOURCE OF STIMULUS

- A. **Extero-ceptors**, located at or near integumentary surface of the body, stimulated by direct effects of *environmental forces*.
 - 1. Pressure receptors of the skin and sex organs.
 - 2. Noci-ceptors of the skin, "pain spots."
 - 3. Temperature receptors of the skin, "heat spots" and "cold spots."
 - 4. Common chemo-receptors of the skin, stimulated by acids and other irritants.

There is some question as to whether these are a distinct type or are merely noci-ceptors.
- Distance receptors.
 - 5. Photo-receptors, retinas of higher animals, "eye spots" and photo-sensitive skin areas of lower animals.
 - 6. Sound receptors, organs of Corti of the ear.
 - 7. Smell receptors, olfactory organs, stimulated by volatile material in amounts so small that these organs are, in effect, distance receptors.
- B. **Proprio-ceptors**, located in the deeper regions of the body, stimulated only indirectly by environmental forces, but excited by *processes within the organism itself*.
 - 1. Pressure receptors of muscles, tendons, ligaments, joints and of blood vessels and other internal organs.
 - 2. Noci-ceptors of internal organs.
 - 3. Pressure receptors of the inner ear (utricle, saccule and semicircular canals) and of statocyst organs. These are gravity receptors and are excited in accordance with the relative positions and the movements of the head.
- C. **Intero-ceptors**, located in the linings of the *digestive system*, stimulated by conditions in this system.
 - 1. Taste receptors. In some animals these are located in various parts of the skin and are extero-ceptors.
 - 2. Receptors, (probably pressure) in stomach. Their excitation by stomach contractions gives "hunger pangs."

3. Unidentified receptors of the mouth and throat supposed to mediate the sense of thirst.

CLASSIFICATION ACCORDING TO NATURE OF STIMULUS

- A. **Chemo-receptors**, especially adapted for excitation by *suitable concentrations of definite substances*.
 1. Olfactory receptors.
 2. Gustatory receptors, "taste buds."
 3. Common chemo-receptors.
- B. **Mechanico-receptors**, especially adapted for excitation by *certain degrees of mechanical pressure or certain frequencies of vibratory impacts*.
 1. Pressure receptors of skin and sex organs and of internal organs.
 2. Gravity receptors of inner ear and statocysts.
 3. Sound receptors of the ear.
 4. "Hunger pang" receptors of the stomach.
 5. Noci-receptors excited by distensions of internal organs.
- C. **Radio-receptors**, especially adapted for excitation by the *effects of radiant energy*.
 1. Photo-receptors.
 2. Heat receptors.
 3. Cold receptors.

Noci-receptors in general cannot be satisfactorily catalogued in this scheme because they are not especially adapted to any one type of stimulus.

Olfactory Receptors.—The human sense of smell is dependent upon a group of highly specialized receptors. These are the cells of the olfactory epithelium in the mucous lining of the nose. (Fig. 108.) Histological studies showing the endings of the olfactory nerve fibers and the location of olfactory epithelium, all indicate that the olfactory receptors have only a limited distribution in the lining of one of the smallest of the nasal cavities, the olfactory cleft. As this cleft is located high in the nose, somewhat out of the main passage for the respiratory currents of air, substances which stimulate olfactory receptors must either be "sniffed" or must diffuse slowly into the olfactory cleft. The portion of the membrane containing olfactory receptors is between six and seven square centimeters in average total area.

Excitation of these receptors occurs through minute and delicate hairs which protrude from the free surface of olfactory cells. These short microscopic hairs are probably always immersed, under normal circumstances, in liquid supplied in the form of mucous secretion from neighboring glands. An odorous substance must go into solution in this liquid before it can excite the receptors. The suggestion has been made that the odorous substance also goes into solution in some component of the receptor hairs. The composition of the latter is known to include fat-like material because the hairs stain black with osmic acid which indicates the presence of unsaturated fatty acids. Volatile substances, soluble in both water and fat, have therefore been regarded as especially likely to excite olfactory receptors. Tests upon a series of alcohols of varying

solubility in water and oil partially bear out this idea but, on the whole, the solubility of the volatile material in oil appears to be a better index of its ability to excite the sense of smell than is its solubility in water. Obviously, non-volatile substances are not odorous; though, if dissolved in a suitable solvent and introduced into the olfactory cleft, they might be excitants. A satisfactory method for testing this possibility has not been found, owing to the delicacy of the olfactory receptors. They appear to be injured by solutions other than that normally bathing them.

The nature of the chemical structure which confers odorousness upon a given substance has not been definitely determined. It is noticeable that certain substances of similar chemical structure have similar smells. All the esters, for example, have a certain similarity of odor. They are said to excite the same modality of sensation. In other cases, however, substances of rather similar structure have entirely different odors. For the most part, the chemistry of olfactory excitation remains to be investigated.

The extreme delicacy of the sense of smell is well known. Numerous investigations have been made to determine the minimum detectable quantity of odorous substances. Quantities that are almost inconceivably small are sufficient to excite the sense of smell in the case of some highly odorous compounds. One of the mercaptans, having an odor similar to that of garlic, will serve as an example. A known amount of this liquid is evaporated in a room of known dimensions and well mixed with the air of the room. This gives a known dilution of the substance in air. With 4.34×10^{-11} milligrams of mercaptan per cubic centimeter of air, this substance is easily smelled. If as is estimated, 50 cubic centimeters of air is the minimum required to carry the odorous material into the olfactory cleft, this observation indicates that 2.17×10^{-9} milligrams ($1/460,000,000,000$ gm.) is detected. This is a quantity far smaller than the minimum amounts detected in the laboratory by so called "very delicate" chemical tests. The minuteness of the quantities that are effective is no indication that olfactory excitation is something different from a chemical reaction. The law of mass action may still be applied

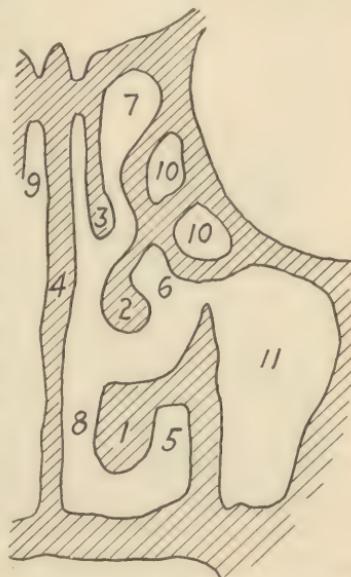


FIG. 108.—Section through the human nasal cavities to show the olfactory cleft. 1, 2 and 3, the nasal septa. 4, septum between the right and left nasal cavities. 5, 6, 7, 8, 10 and 11, nasal cavities and sinuses. 9, the olfactory cleft the mucus lining of which contains all of the olfactory epithelium. (From Parker's "Smell, Taste and Allied Senses," J. B. Lippincott Co.)

to a chemical reaction even though one of the reacting components is in very great dilution. In the experiment just cited, the number of molecules of mercaptan, in 50 cubic centimeters of air, is no less than 200 billions, according to one estimate. The delicacy shown by this and similar experiments upon man is far exceeded by that of the olfactory sense of the dog. In some circumstances, as in scenting game and in following a trail, the dog detects odors that are beyond human recognition. The threshold stimulus for excitation of olfactory receptors must be reached by exceedingly small concentrations in the case of certain odorous substances.

Comparative Physiology.—Olfactory receptors are one of the primitive types of receptor organs. They occur in the lower invertebrates as for example, sea anenomes. In such organisms, these receptors are scattered over the skin area. They are sensitive to very small amounts of food and other sapid substances in the surrounding water. The olfactory receptors are primitive in their general structure in that they have no synapse between the receptor cell and the nerve fiber which connects it with the nervous system. The receptor cells, having direct continuity with the fiber, is thus itself a nerve cell of which the axon fiber is an afferent nerve. This arrangement is distinctly different from that of most receptors for they have a synaptic connection with a true neurone of which the cell body is in a ganglion while in this case, the cell bodies occur in the epithelium. The primitive form of olfactory receptor is found in all animals.

Olfactory receptors play an important part in the tropistic reactions by which animals secure their food. Parker has particularly investigated this type of reaction in fishes. His experiments and those of other investigators have shown that certain species of fishes are largely dependent upon "scenting" of food. When the olfactory organ is destroyed or effectively plugged, these fishes will not take into the mouth a cloth sack containing food as will the normal fish. Copeland has shown that snails also employ the olfactory organs in finding food. Similar tropistic reactions have been discovered in various other forms.

A classification of odors has been attempted on the basis of the human sense of smell. Different groupings have been proposed. A comprehensive one, suggested by Zwaardemacher, gives nine main classes. Among these, are the etherial odors, including those of fruits, the fragrant odors, such as those of flowers, the burning odors, the nauseating odors, etc. Such schemes are not very satisfactory because they depend upon the personal equation. Likenesses and differences among odors are not always similarly recognized by different persons. These classifications are interesting and instructive from the standpoint of general physiology chiefly because they emphasize the undoubted fact that there are distinctly different modalities of the sense of smell. This fact is presumptive evidence that there are different kinds of receptors for olfaction.

Gustatory Receptors.—The receptors for the sense of taste are the so-called **taste buds**. In mammals, these are located chiefly in the tongue but occur scantily in other parts of the oral cavity. The structure of a typical taste bud is shown in Fig. 109. In many fishes, taste buds are not confined to the mouth but occur in the integument of the outside of the body, especially of the head. The catfishes have been studied especially in this connection because they have taste buds in nearly all of the skin area. They are especially numerous upon the barbels or slender tactile processes near the mouth.

The barbels give to several different species an appearance which has led to the name catfishes. One of them, a species of *Ameiurus*, the common bullhead, has been investigated for tropistic responses to gustatory stimuli. This animal responds to the presence of food (chemotropism) even when the olfactory organs are made useless by an appropriate operation. Food substances, such as meat or its juices, can be used as the excitant. When the food is introduced into the water near the animal, it quickly turns so as to bring its mouth into the locality of the food. In the control of this tropistic turning, but even when the food is placed near one side of the tail of the animal, it turns accurately to get the food into its mouth. Tasteless bait, if seen or felt by the fish, may cause it to turn though not in the same way and does not cause it to bite.

Primary Qualities of Taste.—In man, four primary qualities or modalities of taste are recognized, namely, sweet, sour, salty and bitter. Other tastes are believed to be blendings of these primary taste sensations. Very many of the varieties of taste, which we commonly recognize, are really compounded in consciousness out of taste and smell sensations. Some substances which appear to have a characteristic taste, become either tasteless or very different in taste if held in the mouth while the nose is plugged. Onions and apples, for example, cannot be distinguished under these circumstances by taste alone, although their differences in texture may be detected by the sense of touch upon the tongue. The sense of taste is less delicate than that of smell. Alcohol, an example of substances that are both sapid and odorous, is required in larger amounts to be tasted than to be smelled.



FIG. 109.—Taste bud from a papilla of the tongue. Highly magnified. (After Ranzier.)

The Four Kinds of Taste Buds.—Each of the primary taste sensations is probably dependent upon excitation of a special type of receptor. This is shown by the differences in the areas of the tongue that are receptive to the different tastes. The sweet taste is aroused especially on the tip of the tongue though other areas are somewhat receptive. The receptors for the bitter taste are particularly numerous at the root of the tongue though present to some extent elsewhere. Sour and salty taste receptors tend to be concentrated along the sides of the tongue. The taste buds occur for the most part in the walls of the papillae of the tongue. By selecting single papillae and testing each one by single drops of sapid solutions applied with the tip of a fine brush, the receptors of each papilla can be detected. Some of them are thus shown to have receptors for the sweet, or the sour, or the salty taste alone, others for two of the primary tastes, some for three different kinds and a few papillae respond to all four. The failure to find papillae exclusively sensitive to substances that arouse bitter taste, is probably explained by the fact that only the tip of the tongue has been tested in this way. "Bitter receptors" are distinctly congregated near the root of the tongue. No histological evidence or other information is available to show differences in the structure and composition of the four kinds of taste receptors that are assumed to occur.

The threshold stimuli, or minimal concentrations of sapid substances that excite taste buds, are different for the four kinds of receptors. The following table gives some representative results.

Receptor	Substance used	Minimal concentration giving distinct taste	Part of tongue tested
Salty.....	NaCl	0.04 molar	tip and sides
Sweet.....	Sucrose	0.02 molar	tip
Sour.....	HCl	0.002 molar	sides
Bitter.....	Quinine-HCl	0.00004 molar	root

This table shows that sour taste is not a very delicate index of acidity as compared with the action of certain indicators. At $C_R = 10^{-3}$, hydrochloric acid cannot certainly be distinguished as different in taste from neutral water. The sense of bitter is by far the most delicate. The bitterness of strychnine solutions can be detected at even greater dilutions than the minimum required for tasting quinine. As very small quantities of such dilute solutions are sufficient to excite, the delicacy of the receptors for bitter taste approaches that of the receptors for smell.

The process of excitation of gustatory receptors is supposed to occur in the hairlike processes which protrude from the free surfaces of the taste

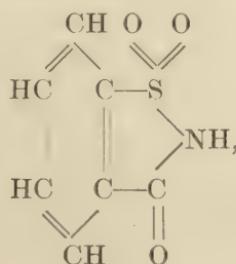
buds. As to the nature of the reactions which occur during excitation, the only observations available are those upon the chemical composition of sapid substances as related to their taste.

Dilute mineral acids possess sourness of taste in proportion to their hydrogen ion concentration and all of these acids, when of the same C_H, are indistinguishable in taste from one another. The hydrogen ion is thus shown to be the exciting agent. But organic acids, notably acetic, possess sourness at dilutions which are high in proportion to the C_H of their solutions. In these cases, therefore, undissociated molecules of the acid appear to influence the excitation.

In the case of salty taste, the anion of the dissociated salt is apparently the exciting agent. This is shown by the fact that, whereas sodium chloride in 0.04 molar solution is distinctly tasted, as are other chlorides in similar concentration, sodium acetate of the same strength and degree of dissociation is practically tasteless. The sodium ion thus appears to be without effect in arousing the sense of taste. The same is true of some other inorganic cations. But all inorganic anions tend to excite the sensation of saltiness. The fact that these same ions do not arouse the saline taste when they are furnished by inorganic acids, is due perhaps, to the more potent excitation produced by the hydrogen ions.

The bitter taste is more nearly characteristic of the alkaloids than of any other group of compounds; but, as it is also given by such entirely unrelated substance as the magnesium ion, the glucosides, and picric acid and as the alkaloids themselves are a very heterogeneous group, no satisfactory conclusions can be drawn as to the chemical structure required to enable a compound to excite the bitter taste. One fact has been well established: When three nitro ($-NO_2$) groups are present in a molecule, the substance has a bitter taste. Picric acid, trinitrophenol, is especially bitter. Many substances with two of these groups in a molecule are bitter, but not all of them. The bitter taste is excited by very diverse substances. Some of them are ions, some are undissociated molecules.

The sweet taste is also excited by substances of very diverse composition. The sugars and their corresponding polyhydric alcohols are prominent; but substances having no chemical relation to the sugars nor to each other, are also sweet. Glycocoll, chloroform, lead acetate, saccharine (a sulpho-benzoic acid imide),



and glucinum ions of the chloride, sulphate, and other salts of glucinum—all of these excite the sweet taste. They are entirely unrelated chemically. The differences in the taste of stereoisomeric compounds is often marked. For example, d-asparagine is sweet, l-asparagine, its optical mate, is tasteless. Sugars that are stereoisomeres vary greatly in sweetness and some of the hexoses are even bitter. These observations lead to no more definite conclusions than do corresponding studies of the bitter taste.

Although the effects of hydrogen ions, of salt ions and of a few groups of compounds have thrown a little light upon the nature of the excitation process in taste buds, this problem is still in a very confused state and is little nearer to being solved than is the problem of the excitation of olfactory receptors.

Common Chemo-receptors.—In addition to olfactory and gustatory receptors, others that are less specialized are sometimes regarded as chemo-receptors. Probably, the skin of all animals is provided, to some extent at least, with common chemo-receptors. Their excitation in the skin of the spinal frog is the cause of the well coordinated reflexes obtained when acetic and other acids are applied to the skin. In human beings, the common chemo-receptors in the mucous membrane are much more easily stimulated than they are in other parts of the integument. These receptors appear to be mere ramifications of "naked" nerve endings. They are excited, in general, by irritating substances, mustard, acids, etc. It is therefore questionable as to whether the common chemoreceptors are different from the nociceptors.

External Pressure Receptors.—The sense of touch is dependent upon receptors which occur in spots on the human skin. This *punctiform distribution* is characteristic of all skin receptors. The actual receptor organs are minute bodies of a bulbar shape forming the afferent nerve endings. The type known as **Meissner corpuscles** (Fig. 110) are abundant in the outer layers of the skin of most parts of the body. Other similar ones occur in deeper layers of the skin. Those found in mucous membranes of the tongue, of other parts of the mouth and of external genital



FIG. 110.—Tactile corpuscle of the skin. Highly magnified.

organs have a characteristic form in each case. The total number of pressure receptors in the skin has been estimated as more than 500,000. Individual pressure spots can be located by application of a fine-pointed instrument. Bristles, the so-called tactile hairs, are useful for this purpose. The subject of the test should not be permitted to

see the tactile hair, lest visual sensations should confuse the judgment of touch. By such tests, the distribution of touch receptors in the skin is found to be very uneven. They are particularly abundant upon the tips of the fingers and the tip of the tongue. Upon parts of the body bearing hair, these tactile corpuscles tend to be more abundant near the roots of the hair than elsewhere. Such an arrangement is an advantage, in that any movements of the hairs stimulate the pressure receptors and thus give added tactile sensitiveness to the skin.

Tactile Discrimination.—Different parts of the skin vary greatly in what is called tactile discrimination, the ability to distinguish two small objects from each other, when they touch the skin at nearby points. An instrument called the **aesthesiometer** is used to test this discrimination. The instrument is a compass with points made of tactile hairs which give uniform pressure when applied simultaneously to the skin. The distance between the tips of the two hairs can be accurately graduated. Tactile discrimination is measured by the distance between the points when it is just sufficient to cause them to be felt as two. Tactile discrimination is approximately the same as ability to judge textures. Obviously, the fingers are more sensitive for this purpose than is the back of the hand, while the tip of the tongue is more sensitive than the fingers, since any irregularity of the teeth feels much more distinct to the tongue than to the fingers.

Some representative figures, showing the results of aesthesiometer measurements, follow:

	Millimeters
Tip of tongue.....	1.1
Tip of finger.....	2.2
Second phalanx of finger, palmar surface.....	4.4
Third phalanx of finger, dorsal surface.....	6.8
Tip of nose.....	6.6
Middle of the palm.....	8.5
Second phalanx of finger, dorsal surface.....	11.2
Forehead.....	22.6
Back of hand.....	31.6
Forearm.....	39.6
Sternum.....	45.0
Region along the spine.....	54.0
Back of the neck.....	67.7

Two points, touching the skin in some regions, must be more than $2\frac{1}{2}$ in. apart to be felt as two points. An explanation of this phenomenon has been suggested in terms of the relative abundance of pressure receptors

in different parts of the skin. But other factors are probably concerned, because the end organs of touch are not sufficiently scarce to account for the low discriminating power of certain areas.

The threshold stimulus for pressure receptors also varies in different areas. It is measured by the pressure in grams per square millimeter that is the minimum required to excite. The threshold stimulus is ten to twenty times larger on the trunk and limbs than it is on the tongue, lips and finger tips.

Plant structures, which closely correspond to true pressure receptors, occur in *Dionaea*, the fly-trap. Hair-like processes on the leaf surface are highly sensitive to touch, so that contact with an insect excites them.



FIG. 111.—Endings of sensory nerves in muscle. Highly magnified.

circular end plates. The end structures of the afferent nerves of tendons and ligaments show similar variations of form. In joints, definite bulbous end organs, like tactile corpuscles, are found. All of these different structures are believed to be connected with afferent nerves because they do not degenerate after the ventral roots of the spinal nerves are cut.

The chief function of all internal pressure receptors is the automatic regulation of muscular coordination as described in the section on the cerebellum. The cutting of afferent nerves from these receptors interferes with muscular control almost as badly as does cutting of motor nerves. But these receptors also have an effect on sensation. This conscious reaction is sometimes called the *muscle sense*. It is also called the *kinaesthetic sense* since it is that which makes us aware of our own motions. A person does not have to look at any part of his body to know whether its muscles are contracted and its joints bent or whether it

Although this excitation is not propagated along definite nerve-like structures, it is conducted to the contractile fibers of the leaf. The flytrap thus shows a reflex with receptor, conducting path and effector.

Internal Pressure Receptors.—The endings of afferent nerve fibers in the muscles are many branched arborizations which, in many cases, extend over the ends of muscle fibers. (Fig. 111.) These terminations are regarded as pressure receptors excited by muscular contraction. Specialized structures other than mere arborizations do not appear to be present in these receptors. The afferent nerves, however, have other forms of endings in the muscles. In some cases they are spirals wound around the muscles. Others have club-shaped terminal organs or tiny

is in motion or in any particular posture. Although the person untrained in introspection may not be aware of it, the kinaesthetic sense keeps him "subconsciously informed" of events in the skeletal muscular system.

Pressure Receptors of the Statocysts and of the Inner Ear.—A primitive form of pressure or gravity receptor, the statocyst, was described above. This organ was formerly called an oto cyst from its supposed functional resemblance to an ear. It is now generally conceded to have no auditory function. The statoliths, which are pressed by gravity against the receptor hairs of this organ were formerly called otoliths or earstones but lacking auditory functions they are more properly called statoliths. They are hard concretions of calcium carbonate in most animals. But in certain species with open statocysts, grains of sand find their way into the cavity and serve as statoliths. This enabled Kreidl to make an interesting experiment on crustacea which, after each molting, acquire new grains of sand to replace those lost during the molt. Freshly molted animals were placed in an aquarium containing iron filings and no other hard grains. After the iron filings had got into the statocyst, the animals were placed in a strong magnetic field. The magnet, acting upon the iron, counteracted the effect of gravity and produced abnormal forced movements of the animals. This experiment thus gave excellent proof that statocysts are gravity receptors. The **saccule** and **utricle** in the inner ear of higher animals are similar organs and serve the same purpose. (Fig. 114.) The hair-like processes which protrude into the lymph within the saccule and utricle are stimulated by the weight of numerous minute concretions of calcium carbonate, which are suspended in this lymph.

An additional mechanism of similar function occurs in the higher animals. This consists of the **semicircular canals** or **labyrinth**. Three of these canals form part of the inner ear on each side of the head. The canals open out of the utricle. Each is a membranous tube lying within a tunnel through the surrounding bone and filled with lymph. The canals are so arranged that each one lies in a plane at right angles to both planes of the other two canals in its labyrinth and is in a plane parallel to that of one canal in the labyrinth of the other ear. (Fig. 112.) Near one end of each canal is a swelling, the **ampulla**. The actual receptor organs are mostly in the ampullae, though they are scattered to some

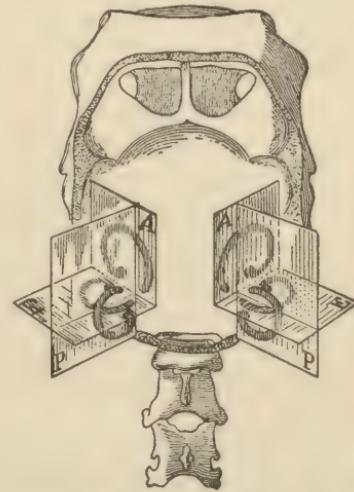


FIG. 112.—Diagram to show the planes of the semicircular canals.
(After Ewald.)

extent along the linings of the canals. These receptor cells bear flexible hairs which project into the lymph. The cells are connected through synapses with fibers of the vestibular branch of the eighth nerve. Bending of the hairs excites the nerves and is produced by movements of the head. Such movements create currents in the lymph of the canals because of the inertia of this fluid. This was proved to be the mechanism

of excitation by experiments made by Ewald. He cut two minute holes into one semicircular canal of a pigeon. He introduced a plug into one hole and an arrangement for exerting pressure into the other. He was thus enabled to create a current in a definite direction in the operated canal. Such currents caused definite forced movements of the bird's head. The arrangement of canals, one on each side of the head in each of the three planes of space results in excitation of one or more of the ampullar receptors during any possible movement of the head.

FIG. 113.—Bird with disturbed equilibrium because of injuries to the semicircular canals. (After Ewald.)

These excitations exert a tropistic control of the muscles, especially those of the neck. The abnormal position of a bird with injured canals (Fig. 113) shows the disturbance of this control when the normal excitation of these receptors is lacking. The bird's partial recovery from the effects of canal injuries is like the corresponding recovery from cerebellar injuries. Improvement is due to the gradual substitution of optical impulses for the missing labyrinthine ones. This is shown by the fact that when the injured bird, having regained postural control, is blindfolded, it is as helpless as when the semicircular canals were first injured.

Excitation of labyrinth receptors affects muscle tonus. This is indicated by the inability of a bird with injured canals to hold the head in a normal pose, especially when a small weight is attached to the head so as to draw it down. Tonus of the muscles of the neck and neighboring regions appears to be abolished.

The chief difference between excitation in the semicircular canals and in the statocysts (utricule and saccule), is that in the former, excitation occurs during movements of the head whether active or passive; while in the latter, excitation is continuous, owing to gravity. Both kinds of



excitation are valuable in the maintenance of body equilibrium through automatic control of skeletal muscles.

Sound Receptors.—Receptors for vibratory pressure waves of the surrounding medium, air or water, are not universally present in all animals. These and the other distance receptors, the eyes, are noticeably more developed in the higher animals than in lower ones. Ears and eyes and the accompanying complexity of organization of reflex paths are, indeed, the chief factors which make one animal "higher," as we say, than another. They give power to explore the environment widely, to discover and obtain food, to have warning of danger before injuries occur and to receive the stimuli which are particularly valuable in enabling the organism to attain a certain degree of dominance over the environment. Their usual position in the head, rather than in some other part of the body, is significant because, during locomotion, the head is the chief exploratory part of the body. The *low threshold* of stimulation and therefore great sensitiveness, the *brief latent period* consumed by the process of excitation and therefore quickness of response, the *large number of individual receptors* in each organ and therefore, fineness of discrimination in hearing and vision—these are factors which render the distance receptors of peculiar value to the organism.

Comparative Physiology of Hearing.—Although sound waves are transmitted through water, most aquatic animals are not provided with typical auditory organs. Fishes are not supposed to have any sense of hearing. They lack a cochlea, the typical sound receptor organ; but the statocyst is a sort of rudimentary ear and, according to Piper, sound vibrations, transmitted through water, arouse electrical variations in the eighth (auditory) nerve of fishes. It is possible that many aquatic forms, including fishes, experience some excitation from pressure waves impinging upon the statocysts and also upon the skin as well. All air-inhabiting vertebrates probably have truly functional ears. A primitive **cochlea** is found in amphibia. Reptiles and birds have a greater development of this organ. In many of them it shows the beginnings of the spiral form, characteristic of its highest development as found in mammals. In the latter the cochlea has one and one half to four spiral turns, giving it a form resembling that of a snail's shell.

The Gross Anatomy of the Ear.—The structure of the human ear is shown in Fig. 114. The external ear includes the **pinna** and the **auditory meatus**. The latter is a tube which terminates at the **tympanic membrane**. This membrane is peculiar in that its shape, distribution of tension and manner of attachment render it *aperiodic*, that is, without any definite period of vibration of its own. It is not "tuned" like a drumhead, but is free to take up any vibrations imparted to it. It is also very effectively damped, that is, prevented from showing after-vibration. This provision avoids the confusion that would result if any

of its vibrations persisted after the corresponding sound waves had ceased.

The middle ear or **tympanum** is an irregularly shaped cavity in the temporal bone and contains the **ossicles** of the ear. The tympanum contains air which should be under the same pressure as the atmosphere if vibrations of the tympanic membrane are not to be so damped as to fail in faithful reproduction of sound waves. The necessary equalization of pressure is insured, under most circumstances, by occasional opening of the **Eustachian tube** which leads from the otherwise closed tympanum

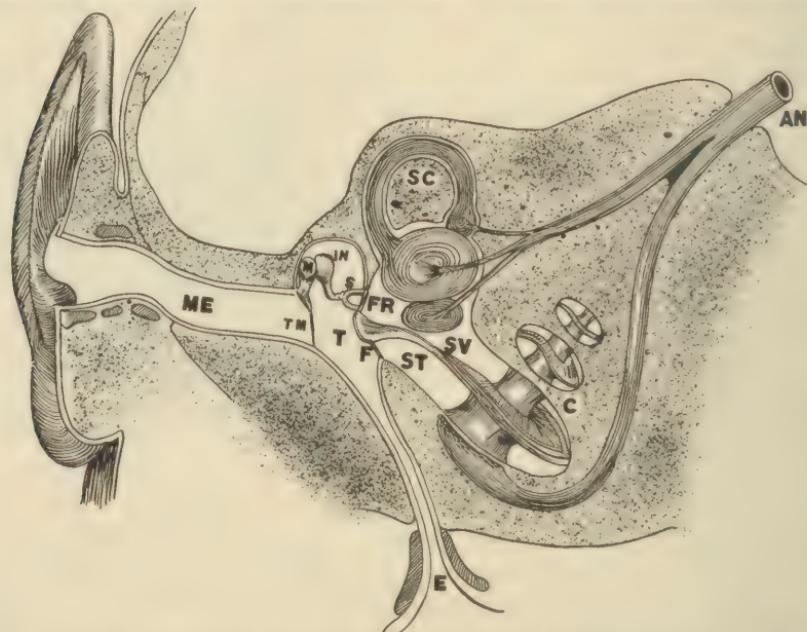


FIG. 114.—Diagrammatic section through a portion of the human cranium to show the parts of the ear. *ME*, external auditory meatus. *TM*, tympanic membrane. *M*, malleus. *IN*, incus. *S*, stapes; *SC*, semicircular canals. *T*, tympanum or ear drum. *FR*, fenestra rotunda. *F*, fenestra ovalis. *ST*, scala tympani. *SV*, scala vestibuli. *C*, cochlea. *E*, eustachian tube. *AN*, auditory nerve. (After Czermak.)

to the pharynx. The Eustachian tube opens during swallowing and this explains why swallowing gives relief from the discomfort and dulling of sense of hearing which follow rapid changes in atmospheric pressure such as occur in going up or down a mountain railway or a mine shaft.

The inner ear comprises the **utricle**, the **saccule**, the **semicircular canals** and the **cochlea**. The latter occupies a spiral shaped tunnel in the surrounding bone.

Transmission to the Cochlea.—The three ossicles of the middle ear transmit the vibrations of the tympanic membrane to the base of the cochlea without change of frequency. The arrangement of the ossicles,

malleus, incus and stapes is indicated in Fig. 114. The stapes is fastened to the membrane which covers the **fenestra ovalis**. The vibrations of this membrane are communicated to the **endolymph** which fills the cochlea. The endolymph is able to take up the vibrations because another membrane over the **fenestra rotunda** bulges out with every inpushing at the fenestra ovalis.

Anatomy of the Cochlea.—The cochlea, throughout the whole extent of its spiral, is divided into two main tubes: The **scala vestibuli**, with its lower termination at the **fenestra ovalis**, and the **scala tympani** with its lower termination at the **fenestra rotunda**. (Fig. 115.)

The structure lying between these two tubes is called the **membranous cochlea**. The lower part of it is formed by the **basilar membrane**. (Fig. 116.) This supports the **organ of Corti** which comprises the

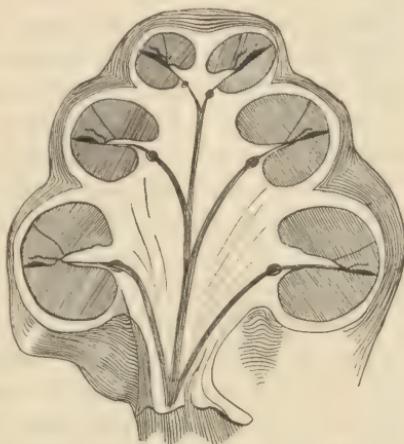


FIG. 115.—Diagrammatic section through the human cochlea to show its coils at different levels, the membranes which divide it and the nerves connected with it.

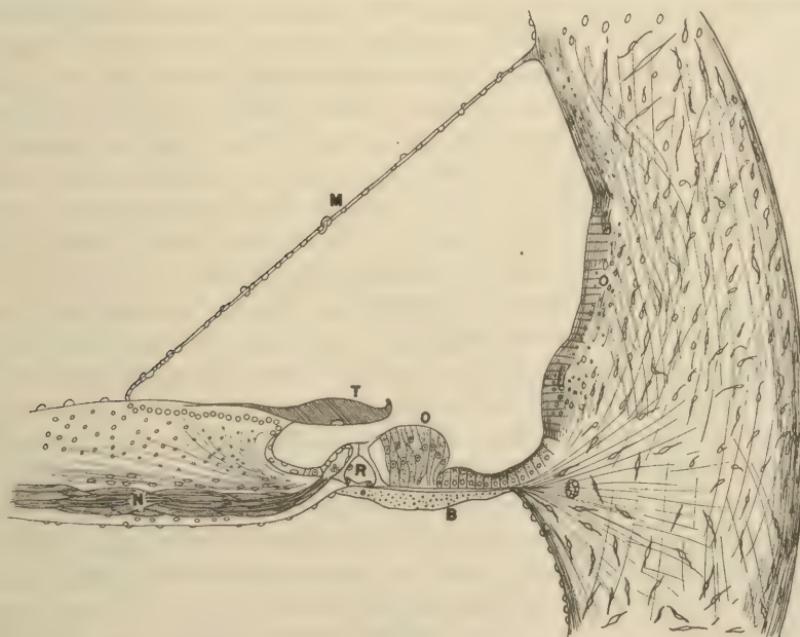


FIG. 116.—Section through the membranous cochlea, highly magnified. *N*, nerve. *B*, basilar membrane. *R*, rods of Corti. *O*, organ of Corti. *T*, tectorial membrane. *M*, Reissner's membrane.

sensory cells that are in connection with the neurones of the auditory nerve, other epithelial cells, supporting cells and the rodlike structures called the rods of Corti. Partly overlapping the organ of Corti is the **tectorial membrane** or membrane of Corti. Above these structures is a small space, the **cochlear canal**. The thin **Reissner's membrane** separates this canal from the scala vestibuli. Inasmuch as the membranous cochlea is in synaptic connection with the auditory branch of the eighth nerve, this part of the cochlea must contain the receptor mechanism.

The Resonance Theory of Auditory Excitation.—The action of this receptor mechanism is believed to be in accordance with the principle of resonance or sympathetic vibration. This is the theory of auditory excitation proposed by the physicist, Helmholtz. The familiar principle of resonance is well illustrated by the behavior of piano strings when a note is sung into the instrument; for under these circumstances, the strings which produce any of the tones or overtones of the note that is sung will be set in vibration so as to give out the tones which they receive. The sympathetic vibrations of the piano strings thus analyze the sound by picking out the different components of the complex air pressure wave. In a corresponding way, some structure of the membranous cochlea is assumed to resonate to the vibratory motions of the endolymph and thus to excite specific neurones of the auditory apparatus in response to a given sound. This theory accounts for the sensory discrimination between sounds of differing pitch, that is, sound waves, of different frequency. In view of the "all or nothing" principle, this theory does not account for discrimination between different degrees of loudness which is dependent upon the amplitude of the vibration of sound waves. Nor does it explain discrimination between tone qualities which are dependent upon the shape of sound waves. Nevertheless the human sense of hearing detects very keenly these three characteristics of any sound: Pitch, loudness and quality. The behavior of animals gives plain evidence that they similarly distinguish different sounds.

The Mechanism of Auditory Excitation.—So far as the theory does account for auditory discrimination, it assumes that different sounds excite different combinations of neurones. The number of fibers in the human auditory nerve has been estimated as about 14,000; so that the different possible combinations of them, which might be simultaneously excited, is practically unlimited. The great question to be answered in connection with cochlear excitation is this: What structures are capable of sympathetic vibration so that their resonance can take up and analyze the complex vibratory waves which are set up in the endolymph? Helmholtz, in his original theory, pointed to the rods of Corti as the resonators. But although these are several thousand in number, they have been reckoned as insufficient to account for the different degrees of pitch of sounds which can be distinguished. Moreover, the rods of Corti are

not present in birds, although these animals clearly distinguish pitch. More recent views attribute to the rods of Corti the function of intensifying the effects of sympathetic vibration upon sense receptors. Another modification of the Helmholtz theory was suggested by Hensen. It assumes the basilar membrane to be the resonator. This membrane is narrower at the base of the cochlea than at the apex. Minute fibers extend across the membrane and are imbedded in it. The fibers vary in length from 135μ at the base to about 234μ at the apex, corresponding to the width of the basilar membrane at each level. These fibers appear to be sufficiently separated from one another to vibrate independently and as one estimate places their total number at about 24,000 in the human ear, there appears to be enough of them to account for the different degrees of pitch that are distinguished. Evidence favorable to this theory was obtained by Baginsky and others from experiments on animals in which the entire cochlea of one ear and part of that of the other was destroyed. With only the base of one cochlea intact, the animal reacts to high pitched sounds while the apex is similarly shown to be sensitive to low pitched ones. The theory assumes that each of the fibers of the basilar membrane has its own period of vibration and can resonate so that the collection of fibers in the basilar membrane can analyze sound vibrations as piano strings do. There are several unsatisfactory aspects of this theory. The fibers are very short to act as efficient resonators, especially in view of the fact that they are not very free to vibrate. Moreover, the theory does not attempt to account for the manner in which specific vibrations in the structures of the basilar membranes are enabled to excite specific sensory cells. These cells are provided with hairlike processes which protrude into the lymph. A further modification of the Helmholtz theory assumes that these processes are the resonators which take up the vibrations of the endolymph and thus excite the sensory cells directly. Still other views attribute the resonating action to the tectorial membrane or membrane of Corti. (Fig. 116.) The free edge of this membrane might presumably vibrate so as to excite the hair cells. Other theories attribute to this membrane the function of dampening after vibrations. Even the fundamental principle of the Helmholtz theory, resonance, has been shown to fail to account for all the phenomena of hearing. The only alternate theory is that which supposes the entire basilar membrane to be thrown into rippling waves. The shape, amplitude and frequency of these minute waves might conceivably vary so as to differently excite the nervous part of sound receptors. This is sometimes called the "*sound pattern*" theory. It is not easily reconciled, however, with the experimental fact that receptors for sounds of differing pitch are distributed along the cochlea.

The conflicting views regarding the mechanism of cochlea excitation are unfortunately very difficult to subject to crucial tests of experiments

and direct observations. Perhaps the nearest approach to a technique for such tests is that used by Yoshii. He subjected each of a series of guinea pigs to hearing continuously a particular loud note from an organ pipe or a siren during 30 to 40 days. The animals were then killed and the membranous cochlea was examined histologically to detect the degenerative effects of prolonged excitation. Distinctly localized degenerations were found in the organ of Corti and were located in different places according to the note which the animal had been hearing. Moreover, the degenerations were transverse, that is, across the organ of Corti, as is required to agree with the Helmholtz theory. Yoshii also found degeneration in the nerve fibers and ganglion cells associated with the particular portions of the organ of Corti affected by the sound.

Photo-receptors.—The majority of animal cells do not respond to light waves of the visible spectrum. The presence of some substance, which is chemically altered by light, is necessary. If any such compound is in the skin, the animal is photosensitive. All pigmented plants are photosensitive because of their content of chlorophyll or related compounds. If a photosensitive material in an animal's skin is nervously connected to muscles, movements result in response to light. The light reactions of sea-anemones (p. 338) and of many other lowly animal forms are of this primitive type. Definite photo-receptors have not been found in such animals. In other animals, slightly higher in the scale of organization, photo-sensitive material occurs in definitely specialized receptor cells which are in effective connection with afferent nerves. Such a mechanism is found in the mollusc, *Pholas*. This animal retracts its siphon in response to illumination. Light acts upon photosensitive material in pigmented cells in the skin of the siphon. These cells contract and by so doing appear to stimulate afferent nerves. The resulting reflex response is contraction of the muscles which draw in the siphon. A similar arrangement is that of the "eye spots" found in many coelenterates and molluscs. In the photo-sensitive spots of an oyster, for example, pigment is so changed by light that a nerve impulse originates in these light receptors. A definite reflex response, closing of the shell, results from this excitation. Such a mere sensitiveness to light is very different from vision. Vision requires the projection of a focused image of the source of light upon suitable photo-receptors. In other words, an eye is required. Primitive eyes, such as those of the scallop (Fig. 117) are probably not very effective optical mechanisms. Although unable to produce very sharply focused camera images, they nevertheless serve to show to their possessor the general outlines of objects, the pattern of lights and shades, and particularly to give warning of the movements of visible objects.

The limited value of a sensitiveness to mere light and shade is seen in the behavior of shell fish. The oyster shows what is called the dark or

shadow reaction. Any shadow, even a slight one, falling upon the photo-sensitive spots or moving across them, causes instant closure of the shell. These spots are located on portions of the oyster mantle which slightly protrude from the gap made when the valves open. The closure is a protective response to the near presence of a potential enemy, for it is more readily obtained by making a shadow move across the mantle than

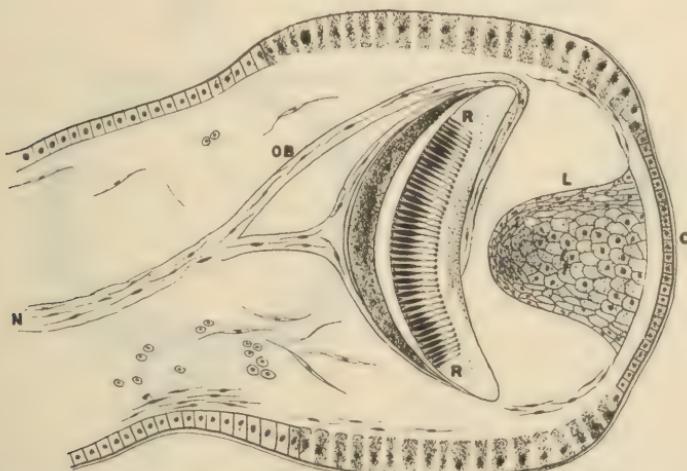


FIG. 117.—Diagrammatic section of the eye of the scallop. *N*, optic nerve. *OB*, outer branch of optic nerve. *RR*, retina. *L*, lens. *C*, cornea. (After Dakin.)

by illuminating it. The scallop, however, possesses the additional advantage of eyes. A number of them are on the edge of the mantle. Each eye has numerous photo-receptor cells, constituting a retina with elaborate nerve connections. Focused images of objects are produced on the retina so that shadows are not required to stimulate. If the visible object moves, thus shifting the images in the eyes, the animal responds by protrusion of its long tentacles in which are the olfactory and pressure receptors. Stimulation of these receptors determines the further responses of the animal, whether closure, flight or feeding.

The Eye as a Camera.—The dioptric mechanism for the formation of visual images is different in different animal forms. The simplest mechanism is that found in many annelids and in the mollusc, Nautilus, which have very primitive eyes built on the principle of the pin hole camera. (Fig. 118B.) Other forms of primitive eyes serve to at least concentrate the light upon the photo-receptors without producing sharp images. (Fig. 118.) The eyes of the peecten (Fig. 117) and of many other higher invertebrates are nearly as complex as those of vertebrates. The compound eyes of insects are made up of a group of photo-receptors rather than unit organs.

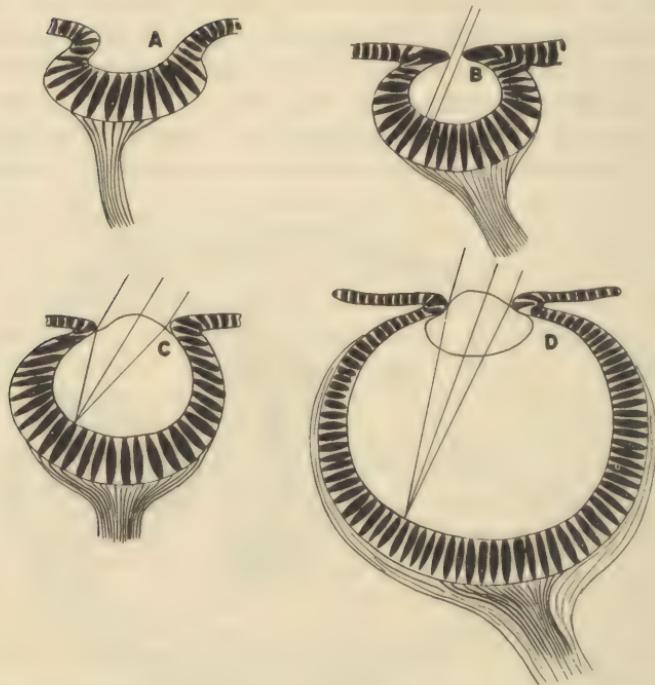


FIG. 118.—Diagram to illustrate primitive forms of eyes. *A*, eye-spot form. *B*, the pin-hole form. *C* and *D*, imperfect focusing forms. (After Starling.)

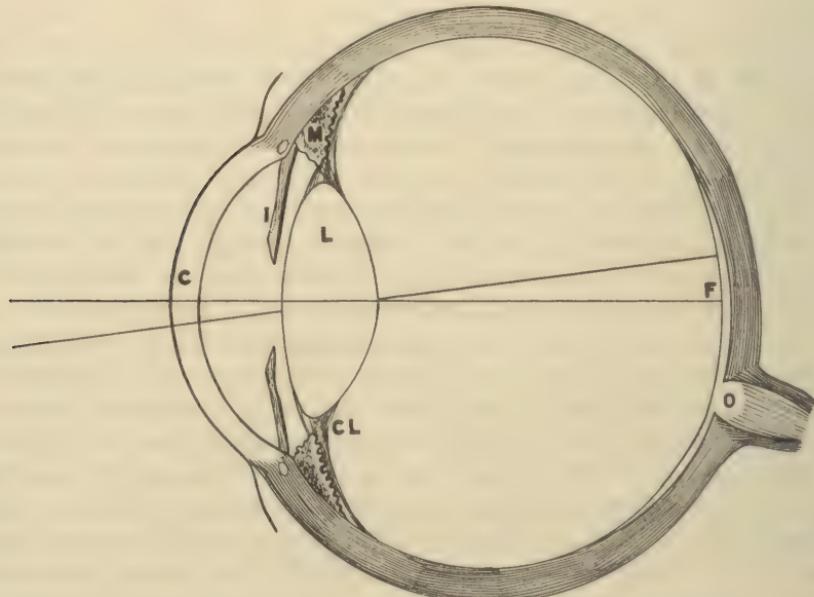


FIG. 119.—Diagrammatic section of the mammalian eye. *C*, cornea. *I*, iris. *L*, lens. *M*, ciliary muscle. *CL*, ciliary ligament. *F*, fovea. *O*, optic nerve.

A resume of the structure of the typical mammalian eye is given in Fig. 119. The tough **sclerotic coat** serves as protection for the eyeball and is comparable to the box of a camera. The **choroid coat** contains most of the blood vessels of the eye and a layer of dark pigment. In some respects, this coat is comparable to the black lining of a camera. The **retina**, containing photo-receptors, is the sensitive film. The **crystalline lens** and the **cornea** furnish the focusing power. The **aqueous humor** in the anterior chamber and the **vitreous humor** in the posterior chamber maintain intraocular pressure and thus rigidly retain the shape of the eyeball. This fullness of the chambers is primarily dependent upon blood pressure in the circulating vessels of the eye and upon the general nutritive condition of its tissues. The **iris** is comparable to the diaphragm of a camera and the **eyelids** to the shutter.

The focusing power of the mammalian eye is not so much dependent upon the crystalline lens as upon the cornea. This is strikingly shown by observations upon the excised eye of the albino rabbit. This eye lacks pigment so that one can see a focused inverted image upon the rear wall of the eyeball when it is held facing a bright object, such as a window. If now, a flat piece of glass is placed in front of the cornea with a drop of physiological salt solution filling the space between the glass and the cornea, the image is no longer seen. Light is not greatly refracted in passing from a water solution to the cornea so that the latter loses most of its focusing power under these conditions. The refracting power of the crystalline lens is thus shown to be insufficient to produce a focus. Fishes could derive little benefit from a curved cornea and indeed they have a cornea that is less curved than that of mammals. Fishes, however, have a nearly spherical crystalline lens, much more curved than that of mammals. The comparative focusing power of the cornea and the lens is accurately shown by physical computations based upon the refractive indices of the cornea, the crystalline lens and the humors the eye.

Accommodation, or adjustment of focus for objects at different distances, is necessary because a sharply focused image must fall upon the photo-receptor cells to produce clear vision. Accommodation is differently produced in different animals. Many animals possess little or no power of accommodation. In fishes, the crystalline lens is moved back and forth by contraction and relaxation of an intraocular muscle, the *retractor lentis*. (Fig. 120.) This changes the distance between the lens and the retina as the focusing arrangement of a camera changes the distance between the lens and the photographic film. In the cuttle fish, *Sepia*, a cephalopod mollusc, the entire eyeball changes its shape during accommodation. Its front is drawn backwards by contraction of intraocular muscles to accommodate for distant objects. (Fig. 120.) Accommodation in mammals is dependent upon the elastic properties of the

crystalline lens. The lens increases the curvature of its surfaces and particularly of its anterior surface when the eye accommodates for vision of near objects. This is proved by watching the mirrored image of a small flame as reflected from the surface of the lens and seen through the pupil of an observed eye. If the subject in whose eye the tiny mirrored image is being observed, changes focus, the images are seen to move in a way that can only be explained by change in the curvature of the lens.

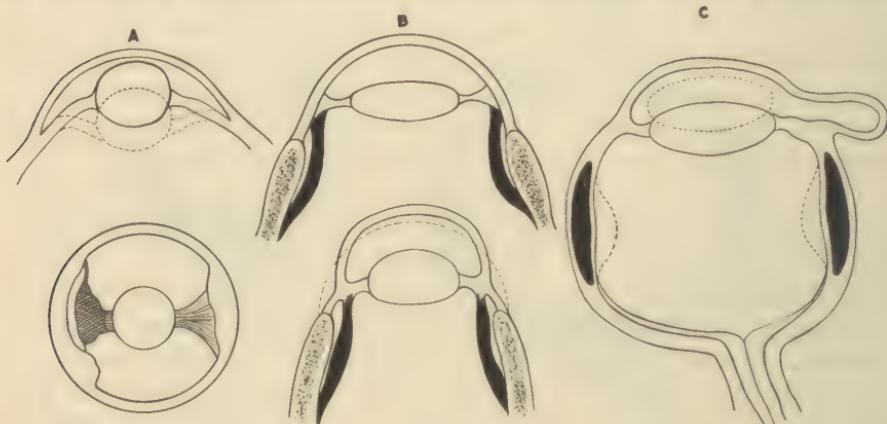


FIG. 120.—Forms of accommodation mechanisms. *A*, in fishes. *B*, in birds. Crampton's muscle shown in black. *C*, in a mollusc eye which changes shape during accommodation. (After Starling.)

The mechanism of this change was satisfactorily explained by Helmholz. The lens is held in a stretched condition by the pull of the **ciliary ligament**. (Fig. 119.) The fibers of this ligament extend radially in every direction from the margin of the lens and are attached to the **ciliary body** which forms a ring around the margin of the lens and is firmly attached to the front of the choroid coat. Intraocular pressure maintains the tension of the ligament so that the lens is in a partially flattened condition when the eyes are at rest. This happens when the eyes are not in use or when they are focused upon objects at a distance of about 20 feet or more from the eyes. At all such distances the light rays, emerging from one point and entering the pupil of the eye, are practically parallel rays and come to a focus in approximately the same plane, namely, the plane of the layer of photosensitive cells in the retina. This layer is that of the **rods and cones** and is about 85μ in thickness. When an object is moved from an infinite distance (parallel rays of light) up to a point about 20 feet from the eyes, its image in the eyes moves back from the lens only a minute distance, less than the thickness of the layer of rods and cones. Accommodation is therefore unnecessary for clear vision of distant objects with any normal eye. Clear vision of nearer objects necessitates accommodation. The ciliary muscle produces it.

The fibers of this muscle are so arranged that their contraction pulls the ciliary body slightly forward. This movement releases the tension of the ciliary ligament. The lens then increases its curvature by its own elasticity. The curvature is proportional to the amount of contraction of the muscle.

The limit of accommodation is the minimal distance between the eye and an object that can be focused. It depends upon the elasticity of the lens. This varies with age so that the near point of distinct vision changes from a little over 2 inches at birth to about 40 inches at the age of sixty. The power of accommodation steadily decreases with advancing age. The age of a person can be told with fair accuracy by measurement of the limit of accommodation. The change in the lens is slow during early life but increases in rate. At about the age of fifty, nearly all normal human eyes are unable to focus for near vision. Glasses of convex curvature are required to aid the focusing power of the eyes for close work. This condition of old-sightedness is technically called **presbyopia**.

The nervous control of accommodation is by means of autonomic fibers in most animals. The ciliary muscle is non-striated in most animals. Accommodation is therefore an involuntary reflex. The stimulus is in some way associated with the action of the muscles which move the eyeballs. The nearer an object, the more must the lines of sight of the two eyes be converged. The convergence is a voluntary act and is dependent upon the contraction of the internal rectus muscles. The development of tension is accompanied by a corresponding contraction of the ciliary muscles. The anatomical arrangement of the nerves controlling these two sets of muscles suggests an explanation of the control of accommodation. The third cranial nerve, the **oculomotor**, supplies the voluntary internal rectus muscle. Some of the fibers of this nerve terminate in synapses in the ciliary ganglion which lies just behind the eye. Some of the post-ganglionic fibers continuing from this ganglion control the ciliary muscles. The corresponding preganglionic fibers have their origin in the brain stem near that of the motor fibers which control the extrinsic eye muscles. The inference is that voluntary control of the extrinsic muscles automatically causes a coordinated control of the ciliary muscles. The act of accommodation and the directing of the line of sight thus constitute an associated reflex. Inasmuch as the ciliary muscle is non-striated and under autonomic control its action would be expected to be involuntary, yet accommodation certainly occurs in response to volition. It is therefore said to be a type of reflex intermediate between the voluntary and involuntary ones. Although not independently under the control of the will, it does occur as an accompaniment to the voluntary act of moving the eyeballs. Even the latter act, although performed by voluntary muscles and under the direct control of central motor neurones, is usually an involuntary reflex.

act. The eyes automatically follow the shifting of the retinal images of moving objects. The visual centers are apparently in functional reflex connection with the motor centers controlling the eyes.

The accommodation mechanism in birds differs from that in mammals. Accommodation might be expected to occur with great rapidity in birds. The gull swooping to pounce upon a small moving fish must change the focus of its eyes very rapidly if the same small object is to be kept in view. Accommodation in birds depends upon the contraction of striated muscle,

under direct control of central motor neurones. This muscle is called **Crampton's muscle**. (Fig. 120.) Voluntary type of control gives greater rapidity of accommodation and probably greater accuracy in birds than in mammals.

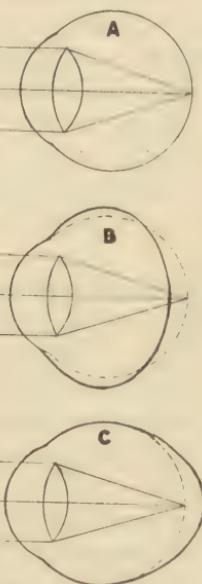
Defects of vision result if the image on the retina is imperfectly focused. This occurs (1) when the shape of the eyeball is abnormal as in nearsightedness, **myopia**, or in far-sightedness, **hypermetropia**; and (2) when the curvature of the refracting surfaces, usually of the cornea, is abnormal as in **astigmatism**.

In the myopic eye, the distance between the cornea and the retina, the antero-posterior diameter of the eyeball, is abnormally great. (Fig. 121.) In rare cases, abnormally great curvature of the cornea or the lens may be the cause. As a result of these conditions, parallel rays of light, such as those coming from objects at a distance of 20 feet or more from the eye, are not focused upon the retina, but come to a focus at a point in front of the retina. Consequently, such a person can see clearly only the objects which are comparatively

FIG. 121.—Diagram of the normal and abnormal shapes of the human eye. A, normal eye in which light is focused upon the retina when parallel rays enter the eye at rest. B, hypermetropic (far-sighted) eye in which the focussing plane for parallel rays is behind the retina. C, myopic (near-sighted) eye in which the focussing plane for parallel rays is in front of the retina.

near. This defect is corrected by wearing spectacles with concave lenses which throw the image further back in the eye. The myopic person without spectacles can see distant objects only as a blur and does not have to accommodate to see comparatively near objects unless they are very near. The myopic person with suitable spectacles sees distant objects without accommodation, as does a normal person, and accommodates for near objects in the normal way.

In the hypermetropic eye, the distance between the cornea and the retina is abnormally short so that parallel rays of light do not come to a



focus upon the retina but tend to meet at a point behind it. Defective curvature of the lens or the cornea is also a rare cause of far-sightedness but in most cases, congenital abnormality in the shape or size of the eyeball is the cause. The hypermetropic person can see objects at a considerable distance by an effort of accommodation but even with the greatest effort, is unable to see nearby objects. Convex lenses which assist the refracting power of the eye are therefore required to correct far-sightedness.

In astigmatism, the cornea is not so curved as to be a portion of a perfect sphere. The commonest cases of astigmatism are those in which the cornea is "spoon shaped" so that its surface is ellipsoidal rather than spherical. Its refracting power is therefore a compromise between that of an ordinary lens with spherical surfaces and that of a lens with cylindrical surfaces. As a result the eye, when accommodated to focus the vertical lines of an object, will not bring its horizontal lines into focus and vice versa. This causes indistinct vision and a constant fatiguing attempt at accommodation. Astigmatism is corrected by astigmatic lenses of curvature similar to that of the abnormal eye but placed with the plane of greatest curvature coinciding with the plane of least curvature of the cornea. If, as is usual, the cornea has greater vertical than horizontal curvature like the bowl of a spoon held horizontally and resting on its edge, then the corrective lenses must have greater horizontal than vertical curvature like the bowl of a spoon held vertically and resting on the tip.

The Accommodation Reflex of the Iris.—In addition to the reflex action of muscles moving the eyeballs and of the ciliary muscle, accommodation also involves a third reflex mechanism. The iris contracts so as to partly close the pupil when the eye accommodates for near vision. The value of this reaction is the correction of **spherical aberration**.

Spherical aberration is the blurring of the image produced by a lens because the focusing power of its central portions is different from that of its marginal zone. This blurring is very noticeable in uncorrected microscope lenses; for if the central portion of the field is in focus, the margins are out and vice versa. This is corrected in optical instruments by the use of a diaphragm which cuts off the light from the marginal part of the lens. The iris fulfills this function for the lens of the eye. Spherical aberration is much more marked with a highly curved lens than with a less curved one and correspondingly, the greater the curvature of the lens, the smaller is the opening of the diaphragm which permits a sharp focus. For this reason, it is an advantage to have the pupil constrict during accommodation for near vision.

Other Functions of the Iris.—The iris also serves to correct **chromatic aberration** which is the production of colored fringes around the edges of a focused image. This is due to the failure of a lens to bring light waves

of different lengths to the same focus. Chromatic aberration is much more marked in the marginal parts of a lens than in its central portions. This fact is easily demonstrated by gazing at a black line on a white background when the pupil is nearly covered so that light can pass through only one side of it. The central part of the lens is thus eliminated. The line is seen with a blue fringe on one side and an orange one on the other. Ordinarily the iris cuts off light from the margin of the lens so as to prevent chromatic aberration even when the pupil is dilated.

The iris is of further value in that it protects the retina from undue exposure to noxious effects of light of great intensity. The constriction of the pupil, so as partly to shut off bright light, furnishes this protection. The reflex control of this reaction is described below.

The Mechanism of the Iris.—The arrangement of the two sets of muscle fibers contained in the iris explains its power to constrict or dilate the pupil. One set of these fibers is circular or ring shaped and the other radial. Contraction of the circular fibers constricts the pupil and contraction of the radial ones dilates it. These muscle fibers are non-

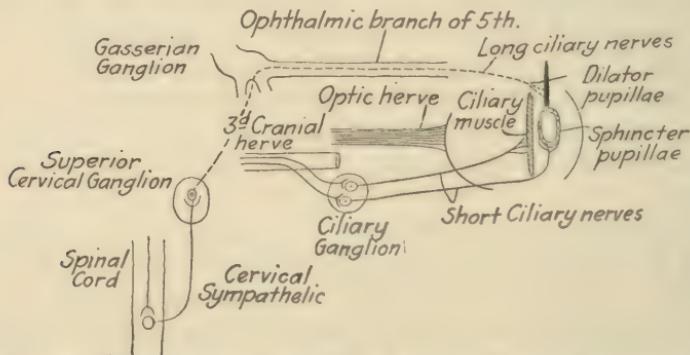


FIG. 122.—Diagrammatic representation of the paths of the pre-ganglionic and post-ganglionic fibers to the ciliary muscle and to the sphincter and dilator muscles of the iris. (After Schultz, from Howell's "Text Book of Physiology," W. B. Saunders Co.)

striated and are under involuntary control through autonomic nerves. Three sets of muscles, (1) the voluntary extrinsic eye muscles, especially the internal rectus, (2) the involuntary ciliary muscles and (3) the constrictor (circular) muscles of the iris, all act in synchrony. They contract nearly simultaneously during the act of focusing upon a nearby object and the tension developed in each set of muscles is roughly proportional to that in the others. All three of these sets of muscles act in an associated reflex manner to produce accommodation.

The nerve supply of the constrictor muscle of the iris consists of post-ganglionic fibers which begin in the ciliary ganglion. The corresponding pre-ganglionic fibers are from the oculomotor nerve as are those for the ciliary muscle and, like the latter take origin in the nucleus of this nerve in the brain. (Fig. 122.)

The nervous control of the dilator (radial) fibers of the iris is entirely separate from that of the constrictor fibers. The post-ganglionic nerve fibers to the dilator muscle originate in the superior cervical ganglion and reach the eye by way of a branch of the fifth cranial nerve.

Apparently, both the constrictor and the dilator muscles of the iris maintain a certain degree of tonus. They are opposing muscles and their action must involve a balanced coordination similar to that of flexor and extensor muscles. Contraction of the dilator muscle has been shown to be accompanied by inhibition of the tonus of the constrictor muscle. The tendency to tonus is probably greater in the constrictor muscle than in the dilator, because the pupil constricts during sleep, although the powerful stimuli for constriction (accommodation and illumination) are lacking. This constriction during sleep does not positively prove the superior tonus of the constrictor muscles because it might possibly be the result of mere inhibition of the dilator fibers. Whatever may be the true explanation, the phenomenon is significant in that it shows reciprocal innervation of non-striated muscle with only one kind of nerve fibers. In many cases non-striated muscles possess both excitatory and inhibitory fibers, but in this case only excitatory ones are present. Inhibition must occur, therefore, in the central nervous system as it does in reciprocal innervation of skeletal muscles. Enlargement of the pupil is obtainable by excitation of the radial fibers, by inhibition of the circular fibers or by a combination of both these events. Correspondingly, constriction of the pupil is obtainable by the reverse of these reactions. As previously explained, this seeming duplication or duality of control gives a high degree of accuracy of graded responses.

The iris muscles also respond to emotional states. The pupil dilates in fright, anger and other emotions. The nervous mechanism of this reaction has not been satisfactorily investigated. Inhibition of the tonic activity of nerve centers controlling the circular fibers might well account for it. This is probably the explanation because any marked cerebral depressions, nervous exhaustion, deep anaesthesia, etc., cause dilatation of the pupil and furthermore experimental stimulation of the cortex of the cerebrum dilates the pupil even when the nerve fibers to its dilator muscles are severed. A similar central inhibitory mechanism is supposed to produce blushing and some other vasomotor responses to emotional states.

The light reflex of the iris is largely independent of its accommodation reflex. In bright lights, the retina is protected from overstimulation by constriction of the pupil, even though the eye is accommodated for distant vision. In dim lights, the pupil dilates. Both the accommodation reflex and the light reflex must employ the same final common path, but they depend upon different stimuli. The stimulus for the light reflex is illumination of the retina. Cutting or degeneration of any part of the

reflex path interferes with the light reflex. Inasmuch as several types of nervous diseases affect parts of the central nervous system lying in this path, failure and abnormalities of the light reflex have become useful signs for diagnosis of these diseases.

The intrinsic photo-sensitiveness of the iris of some animals (p. 334) enables it to respond to light by contracting even when all its nerve connections are severed.

The Rods and Cones as the Photo-sensitive Part of the Retina.—Histologically, the retina is found to be made up of complex nervous structures. They consist of groups of neurones so arranged that their cell bodies and branched processes give the retina a stratified appearance. Careful

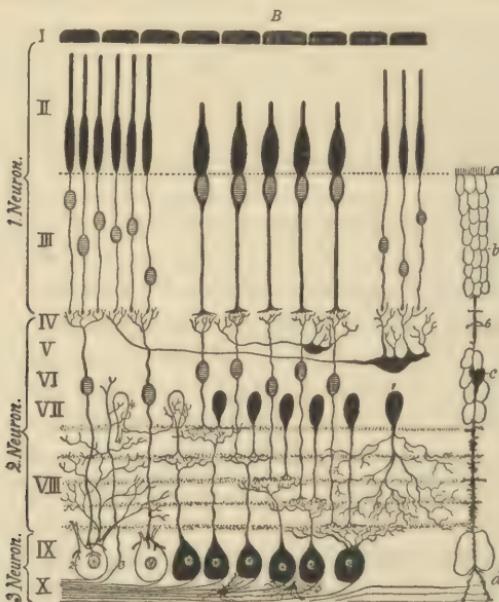


FIG. 123.—Diagram of structures in the human retina. *B*, the pigment layer; Roman numerals refer to the layers or strata seen in histological examination. *X*, fibers which unite to form the optic nerve; fiber of Müller shown at the right. The diagram indicates that at least three neurones must be concerned in the conduction of excitation across the retina. (After Greef, from Howell's "Text Book of Physiology," W. B. Saunders Co.)

analysis of the histological structure of the mammalian retina shows that three sets of neurones must take part, successively, in the conduction of excitation across the retina to the fibers which eventually transmit, by way of the optic nerve, to the brain. (Fig. 123.) Of the complex structures of the retina, only one group, that of the rods and cones, is photo-sensitive. It lies next to the pigment layer which is the outermost one, that is, the one in contact with the choroid coat of the eye. The other retinal structures are the cells and fibers of neurones together with a network of retinal blood vessels. The nervous structures are in functional connec-

tion with the fibers of the optic nerve. These fibers spread out over the retina and form its innermost layer. The retinal blood vessels are all branches of the retinal artery and vein which enter the eyeball through the central part of the optic nerve.

That only the rods and cones are photo-sensitive is shown by the following proofs: (1) When the image of an object falls upon the point where the optic nerve enters the eye, the object is invisible. This is demonstrated by the well known blind-spot experiment illustrated in



FIG. 124.—Blind spot experiment. Close the right eye and fix the left upon the cross. When the figure is held at the correct distance in front of the eyes, the circle becomes invisible because its image falls upon the part of the eye where the optic nerve is connected with the eye. There are no rods or cones in this region. (*From Starling's Human Physiology, Lea and Febiger.*)

Fig. 124. This proves that nerve fibers themselves are not excited by light. (2) In the **fovea centralis** (Fig. 119), on the other hand, is the most acute vision. In the fovea, the retina is thinned to little more than a layer of cones with their attached fibers. This proves that the cones are photo-sensitive. Ordinarily the eyeballs are so moved by their extrinsic muscles that any object to which attention is directed is focused with the center of its image upon the fovea. Indeed, fine details are not discerned for any object of which the image is not upon the fovea. (3) The shadows of the retinal blood vessels are visible under certain circumstances. If the eye gazes through a pin hole in a card held close to the eye, and the card is meanwhile kept in a circulatory motion, one sees a picture of the retinal blood vessels projected upon any brightly lighted area towards which the sight is directed. If gazing at a cloud, for example, one sees it intersected by lines which give it the appearance of a many-branched river system on a map. The apparent size of this system of lines will vary according to the distance at which the judgment places it, but its pattern will always be the same for any one person. It is the pattern of the retinal blood vessels. The same phenomenon is even better shown by the Purkinje experiment. In this, a light, kept in slight circular motion, is held in front and to one side of the eye while gazing at a blank wall of a dimly lighted room. The picture thus produced in the eye is similar to the one just described but is more extended. A third type of observation shows the moving corpuscles in the retinal blood vessels.

Almost everyone has noticed them while gazing at a cloud. They are easily seen while looking into the brightly illuminated vacant field of the microscope. These various observations show that the visual receptors lie back of the retinal blood vessels. The rods and cones are so placed. The ordinary invisibility of these blood-vessel shadows is hard to explain. Apparently such shadows are seen only when made to flicker and thus to fall upon the same rods and cones during only a very brief interval. (4) The rods are shown to be sensitive to light, partly by the fact that they are similar to the cones in location and nerve connections and partly by the fact that the retinas of some animals, for example, bats and owls, have only rods in the layer that is composed of rods and cones in most animals.

Light must pass through the nervous structures of the retina before it reaches the photo-sensitive rods and cones of the vertebrate eye. In the eyes of some of the invertebrates, the cephalopods, the photo-receptors are not placed behind the neurones of the eye but receive the light directly. The nervous structures of this type of eye form a ganglion outside of the eye itself.

Inasmuch as nervous structures compose a large part of the vertebrate retina, the latter is comparable, in a sense, to a little nervous system. The several types of neurones contained in it, are so arranged that a nerve impulse, originating in a rod or a cone, must be propagated through several neurones, probably three, before it leaves the eye by way of the optic nerve fibers. The impulse must cross at least two synapses within the retina itself. The significance of this fact remains to be investigated. It suggests that some correlation of visual impulses occurs in the retina in a manner comparable to the coordinating action of the synaptic central nervous system.

Excitation of the Retina.—The effects of light upon the retina have been studied by contrasting retinas that have been exposed to light with those kept in darkness before the death of the animal. The schematic representation in Fig. 125 shows the changes due to light effects in the frog retina. The following changes are observed: (1) The cones appear to retreat somewhat from the outer layer of the retina. (2) The pigment of the outer or pigment layer moves in between the rods and cones. (3) The pigment of the rods is greatly diminished, that is, bleached by the action of light. The reverse changes: (1) Extension of the cones, (2) partial retreat of the pigment toward the pigment layer and (3) increase in the pigment of the rods, occur when the retina is darkened. Pigment has never been found in typical cones though it is present in the so-called cones of the bird retina. The fovea, containing cones but no rods, is not pigmented. Apparently, the action of light does not cause migration of pigment into the cones. Its movement in between the cones has been observed in the fovea after exposure to light.

Which of these observed changes indicates the mechanism of retinal excitation by light? The movements of the cones probably have no significance in the action of light because this movement is produced by other means, such as injection of strychnine. The movement of the pigment is a comparatively slow process, and therefore, is not a probable cause of excitation. The extension of pigment into the spaces between

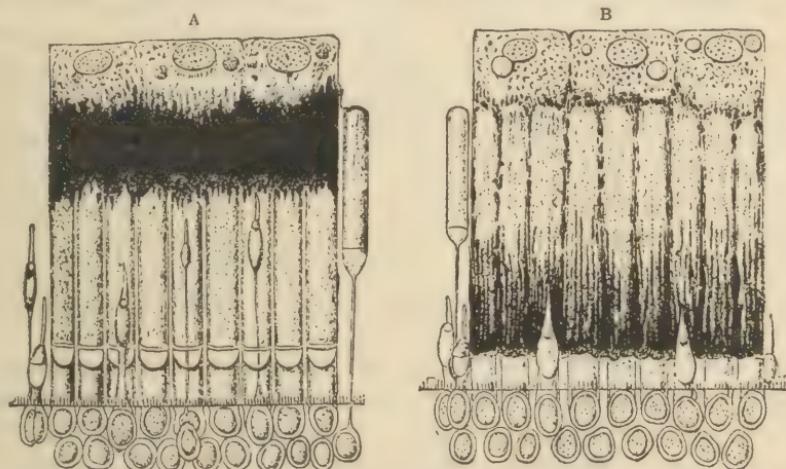


FIG. 125.—Effects of light upon the retina. *A*, after being kept in darkness. *B*, after exposure to light. Explanation in text. (From Burton-Opitz's "Text Book of Physiology," W. B. Saunders Co.)

the rods and cones may possibly be of use in preventing undue diffusion of light from one photo-receptor to another. This movement may also be related to the regeneration of pigment in the rods. Experiments on frogs have shown that when a portion of the retina is detached from the choroid coat and bleached by light, its color does not regenerate until the retina is again placed in contact with the choroid. The pigment coat of the retina is normally in contact with the choroid and apparently must be in this position in order that pigment can regenerate in the rods. The bleaching of the pigment, that is, its decomposition by light, is a typical reaction of a photosensitive substance. It has therefore been especially studied in the hope of explaining the mechanism of retinal excitation.

The pigment of the rods is called the **visual purple** or **rhodopsin**, although its color is more reddish than purple. It can be extracted from retinas of recently killed animals. The best solvent for this purpose, as shown by Kühne, is a solution of bile salts. The latter, as explained above, have marked emulsifying power and are cytolyzing agents, so that they destroy the retinal cells which contain the pigment. Solutions of rhodopsin are bleached by exposure to the light. This bleaching can also be easily shown in the eye itself. For this purpose an albino rabbit is conveniently used. The animal is kept in the dark for a short time

and then killed. Its eye is exposed to a large bright object, such as a window. The retina is then stripped from the eye and treated with alum. A photographic impression or optogram of the window or other object to which the eye was exposed, is the result. Inasmuch as the optogram reproduces the focused image in the eye, the bleaching of rhodopsin by light appears to be in some way concerned with visual excitation. But this explanation is far from satisfactory. In the first place, there is no visual purple in the cones which are the especially sensitive photo-receptors. In them, at any rate, the mechanism of excitation must be something other than a change of rhodopsin. The presence of some other photo-sensitive compound can only be conjectured. Moreover, rhodopsin is not absolutely necessary for vision because it is entirely lacking in the eyes of some animals, including some species of birds and some species of reptiles. The behavior of rhodopsin in the rods of the retinas of most animals has led, however, to the generally accepted conclusion that its decomposition under the influence of light causes excitation of the rods.

The most convincing evidence for this conclusion is found in the study by Henri and des Bancels of the effects of different wave lengths of light upon the eyes and upon rhodopsin. When light is thrown upon the peripheral parts of the human retina, light of any wave lengths between 440 and $600\mu\mu$, blue to orange part of the spectrum, is perceptible, although, as explained below, it gives a mere sensation of light without any sense of color. The minimum amount of energy required to excite sensation, that is, the threshold stimulus, can be calculated for each of these wave lengths. The factors required for this calculation are the candle power of the light and the time that it must act upon the retina in order to excite. When the energy of the threshold stimulus is thus computed for a number of the above wave lengths and is plotted as ordinates against the corresponding wave lengths as abscissas, a curve (see Fig. 126) is obtained. If, now, the energy required for the bleaching effect of light on rhodopsin and also that required for a given degree of light absorption by it are similarly computed and plotted, two curves that are very closely parallel to the first one, are obtained. (Curves in Fig. 126.) These results show that when rhodopsin absorbs light energy in amounts just sufficient to cause bleaching (decomposition), the retinal rods are excited. Decomposition of rhodopsin by light thus appears to be the exciting mechanism in the rods of the human retina.

By analogy, colorless photo-sensitive substances may be supposed to function similarly in the cones and in non-pigmented rods. It must be admitted that this is an unproved deduction and that, in spite of much research on the subject, the nature of the excitatory process in the cones is still conjectural. The possibility that photo-chemical changes of rhodopsin, outside of the rods and cones, might cause excitation upon

the surface films of these receptors does not seem to have been investigated.

Other observations of an entirely different type have been directed toward the explanation of retinal excitation. These are observations upon the electrical variations which occur in the retina when it is illuminated or when light is withdrawn from it. Both illuminating and darken-

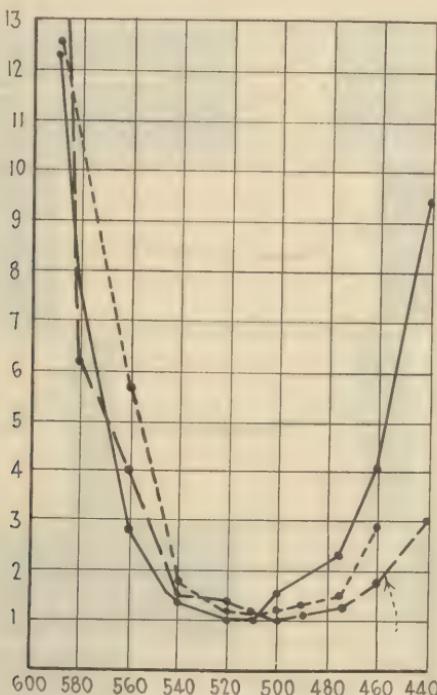


FIG. 126.—Curves to show the parallelism between the effects of light on rhodopsin and on human vision. The abscissas are wave lengths of light in $\mu\mu$ units; ordinates are units of light energy required to produce the various effects. The solid line records the effect on vision (threshold stimulus); the dash line (indicated by an arrow) shows the relative absorption of different wave lengths of light by rhodopsin; the dotted line indicates the relative effects in producing a certain amount of bleaching of rhodopsin. (After Henri and des Bancel.)

ing produce complex electrical variations, more complex in the eyes of some animals than in those of others. String galvanometer records of these effects in the eyes of different species are shown in Fig. 127, taken from the work of Piper. Einthoven and Jolly have used such records to deduce the theory that three different photo-sensitive reactions occur in the retina and that the different velocities of these three are the cause of the complexities of the electrical variations. No satisfactory data have been produced to show what the character of these different reactions may be. The only deduction that can safely be made from these electrical studies is that retinal excitation involves more than one photo-sensitive reaction.

Electrical variations in the optic nerve are of the same character as those in other nerves. These variations occur in the optic nerve when the retina is illuminated and also when it is darkened. That both of these changes should excite the optic nerve is to be expected from the corresponding behavior of the retina, since it is excited by being illuminated and by being darkened.

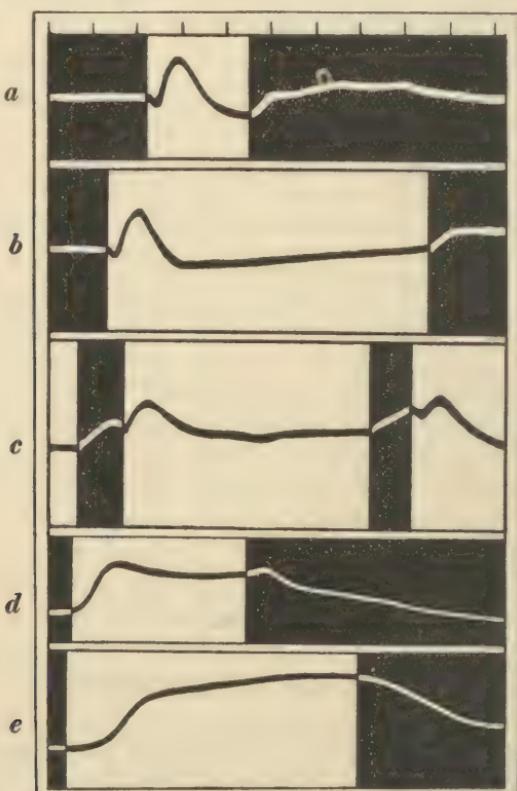


FIG. 127.—Electrical changes produced by light in the retina. The white areas are periods of illumination, the black areas are periods of darkness. Time in 0.1 sec. at the top. *a*, brief illumination of a pigeon eye showing negative and positive variations with light and positive with subsequent darkness. *b*, same with longer illumination. *c*, brief periods of darkening of a pigeon eye after previous, long-continued illumination. *d*, illumination of a rabbit eye showing a positive variation and a slow negative one, after a short latent period, with subsequent darkness. *e*, illumination of a Cephalopod (*Eledone*) eye showing a simple positive variation, after a latent period, and a return to the original value, also after a latent period, with subsequent darkness. (After Piper, from Bayliss, "Principles of General Physiology," Longmans, Green & Co.)

The particular functions of the rods and cones are probably different. The cones are required in order that clear vision of details can be obtained. This is shown by the fact that the image of anything which is clearly seen must fall upon the fovea. The cones are probably best for color vision. This is indicated by the fact that the finest discrimination between the largest number of different colors is obtained only by use of the fovea.

and nearby regions of the retina. The rods, on the other hand, appear to be especially useful for vision in dim lights. The rods show an especially low threshold stimulus. This is assumed to be due to their content of rhodopsin, as explained above. It makes them sensitive to the difference between dim light and darkness even when color and detail are not discernible. In the dark adapted eye, that is, one that has been in darkness or very dim light for a time, the pupil is dilated, so that the retina receives a comparatively large number of any rays of faint light that can enter the eye. Although cones are present in all parts of the retina, those near its outer margins are not typical ones and the rods in this region are more abundant than the cones. This is the part of the retina which is especially useful in the dark adapted eye. The sensitive-

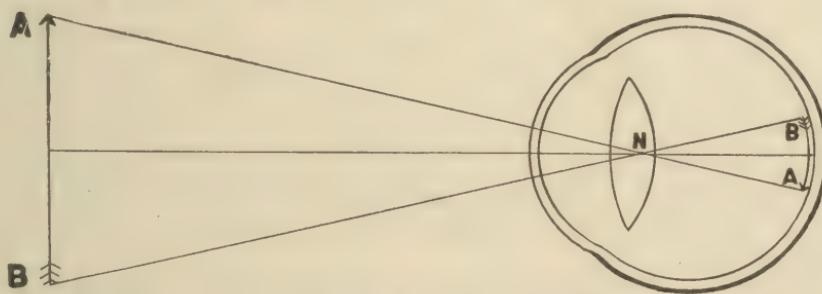


FIG. 128.—Diagram to show the visual angle and the size of the retinal image. The object, $A\ B$, forms the inverted image, $B\ A'$, upon the retina. N represents the nodal point of the refracting surfaces of the eye. If the size of the object and its distance from N are known, the angle, $A\ N\ B$, the visual angle, can be computed by solution of the triangle. The size of the retinal image can be computed by the geometry of similar triangles.

ness of the marginal regions of the retina is easily demonstrated by looking at a very dim star just as it becomes visible at twilight. It is easily seen when the eyes are directed a little to one side of it but disappears from sight if the eyes are turned directly toward it. Its faint luminosity is below the threshold stimulus for the fovea and therefore, presumably below that for all cones, but is sufficient to excite other parts of the retina and therefore, presumably sufficient to excite the rods. The sensitiveness of the marginal portions of the retina increases during the first hour or so of dim lighting, that is, while the eye is yet becoming completely dark adapted. This may be due to regeneration of rhodopsin. The idea that the rods give especial sensitiveness to dim light is further borne out by their predominance in the retinas of night-seeing animals, such as owls and moles.

Visual acuity or distinctness of vision can be measured by finding the diameter of the smallest black circular spot discernible on a white background at a given distance from the eye. Such measurements are usually made with the eyes turned directly toward the test circles, that is, with so-called *direct vision*. The limit of vision is reached when the spot is

so small or is so far away from the eyes that the lines from its margins to the optical center of the eye form a cone with an angle of one minute (60 seconds). By geometry (Fig. 128) the size of the corresponding retinal image can be computed. The smallest discernible image in the fovea has a diameter of about 0.004 mm. (4μ), which is approximately twice the diameter of a foveal cone. In very bright light, the visual acuity may be greater than this. The general conclusion, however, is that at least two different cones must be excited in order that two points may be distinguished from each other. These observations apply only to the fovea. In other parts of the retina, parts which function for the so-called *indirect vision*, the acuity is much less. Here it is necessary that the image in the retina should be at least 0.035 mm. in diameter to be seen. Acuity is thus shown to be only about one-tenth as good in the outer parts of the retina as it is in the fovea. Acuity varies greatly in the eyes of different individuals. This is readily shown by comparison of the limiting distances at which a specimen of print can be read by different persons. Whether this difference is due to the structure of the retina or to other causes, is not known. The visual acuity of many species of birds is assumed to be much greater than that of human beings. Birds appear to see their food at distances which would make it imperceptible to human eyes.

Color Vision.—The physiology of color vision is based almost entirely on the human sense of color. If the image of a well-lighted object falls on the fovea or on nearby points of the retina, all of the spectral colors emitted by the object are distinguishable. This central part of the retina is, therefore, adapted to be differently excited by light of different wave lengths. Parts, farther away from the fovea, fail to make satisfactory distinctions between reds and greens even though the same parts may distinguish yellows and blues. In the outer parts of the retina, excitation produces no sense of color; only light and shade can be distinguished. The different distribution of the receptors for color sense suggests that different photosensitive substances are present in the retina and that each of them enables light of only certain wave lengths to excite the cones. This is the fundamental idea of all the numerous theories which have been proposed to explain color vision. How many such substances exist and how they are affected by specific wave lengths of light are questions which have been variously answered by these theories. Any theory of color vision must take many facts into account. Among them are the following: (1) When blue and yellow light are simultaneously thrown on the same cones, a sensation of white results. Blue and yellow are therefore called **complementary colors**. Similarly, red and green and numerous other pairs of colors produce a sensation of white and so are called complementary. (2) When the eye has been fixed for a few minutes on a bright red object and is then turned toward a white surface,

an **after image** of the same form as the object but of green color is seen. Similarly, after images can be obtained with any color and always show the color which is complementary to the one previously gazed at. (3) Certain color blind persons confuse reds and greens. They judge these colors to be similar to yellow. They distinguish blue but cannot differentiate it from greenish blue or violet. Color blind persons vary as to what colors they judge to be like purple or red. No theory has met all the facts of normal color vision so as to entirely reconcile them to the facts of color blindness. Perhaps the greatest difficulty from the standpoint of general physiology is to reconcile color vision with the theory of unchanging quality of nerve impulses. A given retinal cone appears to be able to excite different color sensations. This implies either that it excites different parts of the visual center or that it starts entirely different qualities of nerve impulses along the same paths. The mechanism, by which a cone might be enabled to do either of these things, remains to be discovered. Descriptions of the theories of color vision may be found in special treatises on vision and in work on psychology.

Color vision is possessed to some extent by animals other than man. Bees and some other insects are shown to distinguish color because they develop a conditioned reflex which makes them turn to a certain color after they have repeatedly found sugar placed upon paper of that color. Birds appear to possess good color vision because they distinguish food by its color. Dogs are said to have at best only a very rudimentary color sense, inasmuch as conditioned reflexes cannot be developed in them, when any one of the majority of colors is used as the associated stimulus.

Binocular Vision.—The value of two eyes instead of one is seen in heliotropism. The significance of the symmetrical, bilateral arrangement of the eyes was explained above in connection with tropisms. Heliotropism is believed to depend solely on the bilateral symmetry of the entire mechanism of photoreceptors, reflex paths and muscles. In animals which do not exhibit heliotropism, binocular vision is useful, as it is in all animals, in extending the visual field. In animals with eyes looking in nearly opposite directions, as in birds (other than owls) and fishes, the two eyes are of obvious advantage in making a large part of the surroundings visible. Owls and many mammals, including man, possess true binocular vision. This is distinctive in that it is stereoscopic. A stereoscopic picture consists of two views taken from slightly different angles. Similarly, the two optical images of a single object are made from slightly different angles. This gives the visual impression of distance and depth as does a stereoscopic picture. It is equivalent to adding a third dimension to the visual field.

The two eyes are reflexly controlled, so that the line of sight of each eye is directed toward the same object. This results in a definite place-

ment of an image of the object in each eye. This placement is such that the two images of a given object lie upon what are called **corresponding points** of the two retinas. Corresponding points are probably in connection with the same neurones in the visual area of the cortex. Excitation of two corresponding points causes only one sensation. This explains why binocular vision is not ordinarily double vision. When the images of a given object fall upon non-corresponding points of the two retinas, the object is seen double. This is proved by pressing one eyeball out of its normal position. Double vision also occurs when the coordinated control of the two eyeballs is defective, as in strabismus, or temporarily lost, as in intoxication. Objects located between the lines of sight of the two eyes can be seen double because their images fall on non-corresponding points of the two retinas. One is not ordinarily conscious of this. Attention is fixed upon the object to which the eyes are directed and for which they are accommodated. But this normal double vision is easily recognized when attention is called to it.

The significance of eye muscle tension for maintenance of equilibrium was indicated above (p. 390). To some extent, the movements which maintain binocular vision produce afferent impulses from extrinsic eye muscles which aid in equilibrium and skilled movements.

Temperature Receptors.—Although studied almost exclusively in human beings, temperature receptors must be present in all animals. They are probably both interior and exterior but only the latter have been well investigated. They occur with a punctiform distribution in



FIG. 129.—Heat spots and cold spots. The skin of a portion of the forearm was tested. Cold spots are plotted at 1, heat spots for the same area at 2.

the skin as do pressure receptors. There are two different kinds, the heat spots and cold spots. (Fig. 129.) The first is stimulated by temperature higher than that of the skin, the cold spots by temperature lower than that of the skin. The threshold stimulus, that is, the degree of heat or cold required to stimulate, varies in different parts of the body. The

receptors of the tongue are especially sensitive; those of the face are less so; those of the trunk and limbs are least sensitive.

The location of temperature receptors can be detected by applying to the skin a fine, blunt pointed object of suitable temperature. The arrangement of the receptors is thus shown to be irregular but is said to be in a pattern or grouping around the hair roots in some parts of the skin. Some parts of the body are sensitive to cold and not to heat.

The structure constituting the receptor is not well understood. Numerous observers have cut out small portions of their own skin containing hot or cold spots and have employed histological methods to

identify the receptor structure. Nothing characteristic has thus been identified. A minute plexus of terminal nerve branches is believed to act as receptor. This has been the general conclusion so far as heat spots are concerned. The structures called Krause corpuscles or bulbs, similar to pressure receptors, are regarded by Von Frey as receptors for cold. But the evidence in favor of this view is not conclusive.

The nature of the excitatory process is probably not specific. Electrical stimulation or even mechanical stimulation can so arouse these receptors that sensations of heat or cold are experienced. Müller's doctrine of the cause of the specific quality of sensations is thus demonstrated. A further illustration of this principle is seen in the so-called paradoxical stimulus. A warm object (about 60°C.) applied to a cold spot gives a sensation of cold. Similarly, parts of the body, supplied with cold spots but no heat spots, are excited to give a sensation of cold when plunged into hot water. Menthol gives a sensation of cold. Carbon dioxide gas, at body temperature, gives a sensation of warmth when the arm is plunged into it. These two cases and some similar instances of chemical stimulation of temperature receptors have not been explained. The well-established fact that CO₂ readily penetrates all cellular structures and thus affects the condition of their buffer substances is significant, since indifferent gases, such as the air, do not affect the temperature sense unless they are distinctly warm or cold.

Temperature receptors probably do not exist in structures other than the skin and mucous surfaces. Proof of their existence in the internal organs has not been shown by either physiological or psychological methods. Although introspection shows that we sometimes refer temperature sensations to the interior of the body, this is not proof of the existence of internal temperature receptors. Indeed, it sometimes happens that a person claims that he is cold or hot when the temperature of the blood and presumably of all internal organs is normal. Moreover, a fever temperature sometimes gives the sensation of being cold when accompanied by chills. The reason for this confusion of the senses is twofold: (1) External excitation by temperature conditions is apparently projected to the exterior only when accompanied by pressure effects, as when a cold or hot body touches the skin. If a warm or cold gas comes in contact with the skin without exerting pressure, it causes an interior projection of the temperature sensation. (2) The presence of an unusual abundance of blood in the vessels of the skin excites a sense of warmth, projected sometimes to the skin, sometimes to the interior; the partial withdrawal of blood from the skin circulation excites a sense of cold, which may also be referred either to the exterior or the interior of the body.

The Internal Senses.—The internal senses are those referred by consciousness to causes within the body, the sense of pain, of internal

temperature, of muscular contraction and of position were discussed in connection with their respective receptors. Other internal senses are those of appetite, hunger, thirst and fatigue. These are not dependent upon the excitation of any particular receptors that have as yet been identified.

Appetite.—It appears to be necessary to distinguish physiologically between hunger and appetite. The nature of the latter has not been subjected to careful physiological analysis. It is known to be excited by the odor and sight of food and probably by the thought of food. Appetite is thus shown to be concerned with conditioned reflexes and may be in no way dependent upon specific receptors of its own. That appetite is also connected with the taste of foods seems probable because those that appeal to the individual taste, tend to especially arouse what we call appetite. Hence the use of appetizers at the beginning of a meal. But the acceptability of food is probably determined more by its previously experienced effects upon the general nutrition of the body than by its more direct effects as excitant of smell and taste. This is indicated by the refusal of animals to continue to eat a monotonous, inadequate diet. Voracious animals, rats, mice and dogs appear to be totally unable to eat such a diet after they have received no other food during several weeks. Gelatin, an inadequate protein, is then refused although eaten with gusto by a normally nourished animal. Gelatin has good nutritive value if eaten along with other proteins that are adequate (p. 30). A diet markedly deficient in either vitamin A or vitamin B causes loss of appetite and tendency toward voluntary starvation. Human beings are apt to experience a positive aversion for a monotonous diet if its nutritive values are ill balanced. The dependence of appetite upon the general metabolism is further shown by the fact that the amount of muscular exercise taken largely determines the appetite. The manner in which nutritive conditions of the body affect appetite, is not known. Loss of appetite is called **anorexia** when it is a symptom of disease.

Hunger has come to be regarded physiologically as distinctly different from appetite. Hunger is a disagreeable if not really painful sensation, while appetite is a pleasurable feeling. Hunger in decerebrate animals is accompanied by restless, almost unceasing movements, indicating a stream of afferent impulses coming into the central nervous system. The "pangs of hunger," experienced by human beings, show one source of these impulses. Cannon found that vigorous contractions of the stomach walls occur just at the time each "hunger pang" is felt. His method for showing this is simple though somewhat heroic. The subject of the experiment, having eaten no food for about twenty-four hours, swallows a thin walled collapsible rubber balloon attached to the end of a rubber tube. The balloon is then blown up through the tube and the other end of the tube is attached to an air pressure recording device.

Any contractions of the stomach increase the air pressure in the balloon and so move the pressure gauge. A writing point, attached to the latter, bears upon a kymograph so that a record of the stomach contractions is obtained. The recording apparatus is screened from the sight of the subject of the experiment. His record of the times, when "hunger pangs" are felt, coincide with the record of the occurrence of violent stomach contractions. Carlson obtained the same result by the use of slightly different methods. He used as the subject of experiments a man with a gastric fistula, a permanent opening from the stomach to the outside of the abdomen. The fistula operation had been necessitated for this patient by an accidental occlusion of the oesophagus. Through the fistula, gastric contractions were observed when hunger pangs occurred. The unpleasant aspect of the hunger sensation does not persist during prolonged starvation. The majority of persons who have experienced starvation testify that no discomfort whatever is felt after the second day of abstinence from food. Even when near a fatal termination, starvation is said to be painless. Only a recently emptied stomach is the source of painful sensations of hunger. The receptors for this sense are apparently excited by pressure. Their identity has not been established. They may be of the same character as the general interoceptors, like those concerned in the pain of intestinal cramps.

Thirst.—The sense of thirst is generally referred in consciousness to the lining of the throat and pharynx. At least, this is true if thirst is accompanied by drying of the mucous membrane in those parts. Thirst of this sort is relieved by moistening the membranes even when no water is swallowed. No receptors excited by dryness are known. Thirst is, however, like appetite, dependent on the general condition of the body. The thirst which follows loss of water, as in sweating, and the extreme thirst which accompanies a high rate of urine secretion, as in diabetes and in the disease called polyuria, show that the sense of thirst operates to maintain the uniformity of the water content of the body. No mechanism, by which the proportions of water in the body fluids could affect the nervous system, has been discovered. Unlike hunger, thirst does not abate during continued lack of water but becomes more painful and is apt to lead to hysteria or other mental disturbances.

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CHAPTER XVII

DIGESTION

Foods that are available for animal uses are rarely in a form suitable for animal metabolism. Proteins must be split into their unit "building stones" in order that the latter may be available for uses which include the synthesis of those proteins that are characteristic of the different tissues of each species. Carbohydrates, especially those of the polysaccharide type, must be split to their constituent monosaccharides before they are efficiently used in animal tissues. Fats need to be hydrolyzed into their constituent fatty acids and glycerol in order that the animal may use these products effectively and particularly that it may construct storage fat of its own peculiar quality. Moreover, the bulk of the food supply of animals is not in a soluble form and must be rendered soluble before it can be utilized. The process of digestion is also required for the separation of useless from useful constituents. All this involves the action of numerous different hydrolyzing enzymes furnished by the digestive juices which act upon the food in the alimentary canal. This action really occurs outside of the body proper; for the digestive system is, in one sense, an infolding of the integument. An animal has been likened to a two-walled sack of which the skin is the outer wall and the lining of the digestive system is the inner wall, continuous with the outer. In the space between these two walls is the living animal. Any process of separation of useful nutrients from their naturally accompanying useless material is, in a sense, a part of the digestive process. All food preparation, including cooking, is a preliminary to animal metabolism, in that it assists the digestive process. The reason why bacteria and all typical plants do not require digestion as a preliminary to metabolism is merely that these organisms utilize foods of so simple a structure that further reduction is not necessary in most cases.

Intracellular Digestion.—The process of digestion, in what might be called a rudimentary form, occurs within the living cells of certain lower animal organisms. In the amoeba, whole organisms, such as unicellular plants, and comparatively large organic fragments are utilized as food. The amoeba, after ingesting such food, produces a temporary organ in the form of a vacuole around this foreign material. Appropriate enzymes appear to be secreted into the contents of the vacuole. At any rate, the foreign matter is visibly altered and partially dissolved. The resulting

products nourish the amoeba. A similar process occurs during phagocytosis in the white corpuscles of higher animals. In them, this appears to be a survival of the primitive intracellular digestive mechanism.

In the sea-anemone the digestive process shows a transitional stage between the primitive, intracellular type and the more specialized extracellular type. This animal has a body cavity which appears, to casual observation, to be like a digestive organ. It contains much sea water which would probably render enzymes ineffective by dilution if they were secreted in the form of a digestive juice. Many long filaments extend into the cavity. These become attached to the fragments of animal and plant structures that serve as food for the sea anemone. Gradually these foods are disintegrated into small masses, possibly because enzymes are produced locally at the points when the filaments are in contact with the food. The small fragments are then engulfed by the cells which line the cavity of the sea-anemone and are subjected to intracellular digestion as in the amoeba.

The Vertebrate Digestive System.—Many variations in the details of the digestive mechanism are shown by the study of comparative physiology of digestion in the vertebrates and the higher invertebrates. But the working of the digestive system in man and the mammals commonly used in laboratory studies typifies that of all these digestive processes. The study of vertebrate digestive systems naturally falls into three main divisions: Digestion in the mouth, including salivary digestion, digestion in the stomach and intestinal digestion.

The Salivary Glands.—The typical arrangement of salivary glands consists of three pairs: The parotids, the submaxillaries and the sublinguals. Saliva is delivered from these glands to the mouth cavity by way of ducts. In addition to the salivary glands, numerous so-called buccal glands secrete into the mouth cavity. The product of these glands is chiefly mucous material.

The Process of Secretion.—The activity of the salivary glands is typical of that of glands in general and is particularly representative of the behavior of digestive glands. The arrangement of gland cells shows an adaptation to the secretion process. Small groups of cells are arranged about a central tubule (Fig. 130) to constitute what is called an *ascinus*. In many *ascini* the gland cells are in a single layer. In any case, a comparatively thin layer of cells composes the *ascinus*. Each *ascinus* is surrounded by lymph spaces and a network of blood capillaries. From the blood, by way of the lymph,

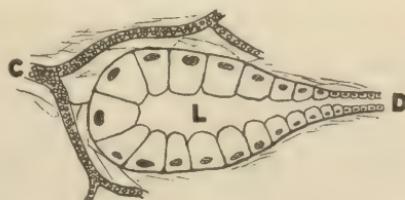


FIG. 130.—Diagrammatic section of an *ascinus* of a gland. *C*, blood capillary. *L*, lumen of the gland, surrounded by a single layer of secreting cells. *D*, duct. Thin fibers of connective tissue form the lymph spaces which surround the capillary and the gland.

abundant supplies of oxygen, inorganic salts, water and the organic nutrient materials required for the secretion process can gain access to the surfaces of the secreting cells that are in contact with the lymph spaces. The product of secretory activities must leave the cells through their surfaces which are next to the central tubule of the acinus. From the tubule, the secretion passes to branches of the duct of the gland and so is delivered by way of the duct.

The changes in secreting cells during activity cause them to appear characteristically different, histologically, in accordance with the amount

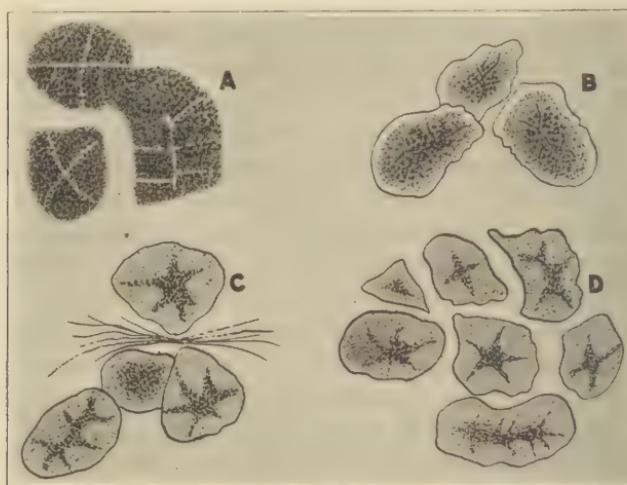


FIG. 131.—Cells of salivary gland after rest and after activity. Sketches of secreting cells of the parotid of the rabbit. *A*, in resting condition. *B*, after secretion caused by pilocarpin. *C*, after more copious secretion induced by pilocarpin and excitation of sympathetic nerve. *D*, after long-continued excitation of sympathetic. (After Langley, from Howell's "Text Book of Physiology," W. B. Saunders Co.)

of secretory activity of the gland just prior to the death of the animal from which the gland was removed. Figure 131 shows the appearance of the cells of salivary glands in the resting stage and after much secretory activity. In the resting stage, the cells are somewhat distended, are filled with highly granular cytoplasm and have the nucleus crowded near to the cell wall. But when the gland has been made to secrete actively the cells are found slightly shrunken. The granules are greatly diminished in number or have all disappeared and the nucleus is nearer the center of the cell. The disappearance of the granules during activity suggests that they are the source of some of the materials which go into the secreted juice. Inasmuch as enzymes are notable constituents of digestive secretions, the granules of the resting cell have been named **zymogen granules**. It is not really known whether or not they are precursors of the enzymes but the granules are colloidal precipitates which are stored up in the cell at rest and are in some way broken down and

washed out of the cell when active secretion occurs during excitation. The nature of the chemical processes in secreting cells can only be surmised. Evidently, the cells take up nutrient material from the surrounding lymph at all times. The cells are minute chemical laboratories in which synthetic processes elaborate new compounds out of the common food supply for all cells, but each secreting cell is able to synthesize some products that are characteristic of its type. At the time of excitation of the cell, a stream of water is set flowing through it from the lymph to the tubule of the gland and processes which involve breaking down of cell granules also occur. The flow of water through the cells removes the product of this breakdown. The forces of osmotic pressure are doubtless concerned in the movements of water; but an explanation of the tendency of these movements to occur in one direction, namely, toward the central tubule, is required in order to explain the secretion process. This explanation has not been obtained satisfactorily.

The Control of Secretion.—Excitation of the salivary glands depends upon nerve impulses under all normal conditions. Only the action of pilocarpine and a few other drugs which, like it, locally excite secreting cells can take the place of nervous excitation to stimulate salivary secretion. The dependence of salivary glands upon the nervous system is shown by the fact that they are paralyzed, permanently and completely, after their nerve connections are cut. The nervous control is reflex. The stimuli which ordinarily arouse the reflex secretion of saliva are: (1) Tasting and smelling of food or of some other substances such as acetic acid (excitation of chemo-receptors); (2) the chewing of foods or even movements of the jaws or the presence of solid substances in the mouth (excitation of pressure receptors); (3) the sight or even the thought of good tasting food. The latter type of excitation is called the **psychic reflex**. It might better be called a **conditioned reflex** as it depends upon previous experience and the formation of reflex associations. Artificial stimulation of the efferent nerves leading to the salivary glands can also effectively excite their secretory activity. This is most easily demonstrated by stimulating the **chorda tympani** with electrodes from an induction coil. As long as the induction current excites this nerve, saliva flows from the duct of the submaxillary gland. A canula inserted in this duct, so as to constitute a salivary fistula, is convenient for observations of the flow of saliva during such experiments. By somewhat more difficult operations, nervous control of the parotid glands can be demonstrated. The salivary glands have a double nerve supply. For the submaxillary glands, for example, sympathetic nerves are supplied in addition to the **chorda tympani**. Curiously enough, both of these nerves are excitatory for the gland but the saliva formed during excitation of the sympathetic nerves is distinctly different from that formed during excitation of the **chorda**. The sympathetic saliva in the dog is much thicker, more viscid

and smaller in amount than the chorda saliva. Some investigators have claimed that stimulation of the sympathetic nerves actually inhibits secretion, when it has been previously excited through the chorda.

Theory of Trophic and Secretory Fibers.—Inasmuch as the sympathetic stimulation does slow down salivary secretion when it is proceeding at a rapid rate and produces a concentrated saliva at all times, Heidenhain suggested that the effect of the sympathetic nerve impulses favored anabolism in the salivary cells. He therefore proposed to call these nerves "trophic" fibers. These are supposed to excite the building up of substances peculiar to the gland cells but to have little or no effect in causing the flow of liquid through the gland cells into the duct. The fibers of the chorda, on the other hand, are the ones which excite copious saliva secretion in the dog. They are supposed to cause the gland cells to set up a flow of liquid from the blood and lymph into the duct of the gland. This liquid carries the product of the cells out of the gland in more diluted form than that resulting from the excitation by "trophic" fibers. Both "trophic" and "secretory" nerve fibers are thus regarded as excitatory but in different ways. Heidenhain's theory is helpful in explanation of the process of secretion but it necessitates some unproved assumptions. The disposition of "trophic" and "secretory" fibers in the nerve trunks must be assumed to vary in different animals, because in some dogs, the sympathetic saliva is not very different from the chorda saliva and in all cases, some flow of saliva is obtained during stimulation of the sympathetic nerves. This is interpreted as signifying that some dogs have numerous "secretory" fibers along with the "trophic" ones in the sympathetic and that all dogs have a few "secretory" fibers in these nerves. But in cats, effects are just the reverse of those obtained in dogs. The sympathetic saliva is more watery than the chorda, so that "trophic" fibers must be assumed to predominate in the chorda.

Inhibition of salivary secretion can be easily demonstrated. The effects of emotional states in causing dryness of the mouth by a stoppage of salivary secretion, are familiar. This inhibition is probably dependent upon a central nervous mechanism. The center for salivary secretory reflexes is located in the medulla. This, like other reflex centers, can be inhibited. This furnishes an adequate explanation of salivary inhibition in spite of the fact that all nerves to the salivary glands have excitatory effects. The center for salivary secretion is probably in a state of mild excitation nearly all of the time. This appears to be the case because enough saliva is secreted to keep the mouth moist even when the stimulus of eating is not present.

The Composition of Saliva.—Normal human saliva can be collected while chewing paraffin, which is insoluble but has the texture of food. Such saliva contains about 99.5 per cent of water. Saliva collected under other circumstances, as when dry and gritty materials like sand

are in the mouth, is less watery. **Mucin**, the chief solid constituent, varies correspondingly. It is less abundant in the more watery saliva. Mucin is a glycoprotein. Its solutions have a high viscosity. This property of mucin confers upon saliva its stringy consistency and makes the saliva, secreted when sand is the stimulus, particularly viscid and of better lubricating quality. Other substances dissolved in saliva include inorganic salts. Chlorides, carbonates, phosphates and sulphates of sodium, potassium, calcium and magnesium can be identified. These are the salts commonly present in all animal fluids. As explained in Chap. V, sodium chloride is by far the most abundant of these salts. The saliva also contains minute traces of numerous organic compounds, for example, urea. These apparently escape from the blood by a process akin to filtration through the salivary gland. Foreign substances, such as drugs, iodides or bromides, similarly "leak out" into the saliva, after they have been introduced into the blood. The tasting of morphine after its subcutaneous injection, is frequently experienced. These facts show that the process of secretion may serve incidentally as an aid to the excretion of wastes and foreign substances. Human saliva contains two enzymes, **ptyalin** or **salivary amylase** and **maltase**. The reaction of saliva when exposed to air is normally on the alkaline side of neutral ($\text{pH} = 7.2$ average). This faint alkalinity is due to the carbonates and phosphates of the saliva. Saliva collected in such a way as to check loss of CO_2 to the air, is normally on the acid side of neutral ($\text{pH} = 6.6$ average). This concentration of hydrogen ions is close to the optimum for the activity of salivary enzymes.

The Functions of the Saliva—The action of saliva on starch was described above (p. 108). Salivary amylase transforms starch through the stages of soluble starch, amylodextrin, erythrodextrin and achroodextrin into maltose. The latter is transformed by maltase into glucose. In addition to starch, glycogen is hydrolyzed into glucose by salivary amylase. Carbohydrates other than starches, dextrins, glycogen and maltose are not hydrolyzed by the saliva. Starches in the form of the natural, unruptured grains, that is, raw starches, are almost unaffected by salivary amylase. This is supposed to be due in part to a thin coating of resistant cellulose which covers the starch grain. The celluloses all resist salivary action. The restricted powers of salivary enzymes illustrate the specificity of all enzymes.

The salivary enzymes are markedly inhibited by a hydrogen ion concentration represented by $\text{pH} = 5$. At $\text{pH} = 3$, they are irreversibly destroyed. On this account, salivary digestion of starches cannot continue after food has been in the stomach long enough to be acidified by the gastric juice.

The functions of the saliva include its action as a solvent. This is particularly valuable because it dissolves enough of food materials to

enable them to excite the taste buds. The taste of food has indirect stimulating effects upon the entire digestive process. Another use of the saliva is the lubrication of food during swallowing. In animals which bolt their food or which consume coarse, dry foods, this is an important function of the saliva. The carbohydrate digesting action of the saliva is its only contribution to the hydrolysis of food in preparation for metabolism. Even this action of the saliva is of minor importance and does not occur in some animals. In the dog, for example, salivary amylase is entirely lacking.

Deglutition.—The act of swallowing or deglutition begins under voluntary control; but after the food has passed over the epiglottis and into

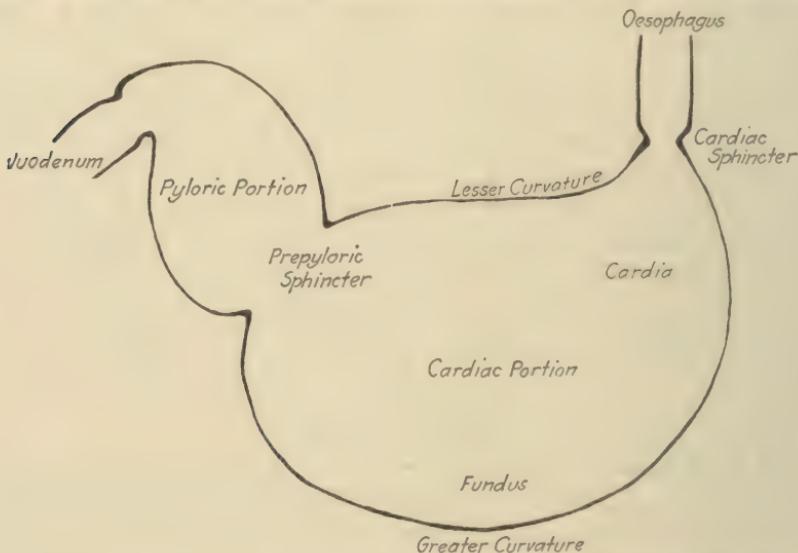


FIG. 132.—Outline of stomach to indicate the chief anatomical features.

the upper part of the oesophagus, it is carried on to the stomach by involuntary reflex action. Circular muscles in the walls of the oesophagus contract so as to produce a **peristaltic wave**. In this, the circular muscle fibers, at any level of the oesophagus, contract just after those which are nearer to the mouth. The peristaltic wave is preceded by a wave of relaxation or inhibition of the tone of the oesophageal muscles. This relaxation also involves the thick ring of muscle called the cardiac sphincter which marks the opening into the stomach from the oesophagus. Ordinarily, the tonus of this sphincter keeps the entrance to the stomach closed. Its relaxation, when the wave of oesophageal inhibition reaches it, opens the stomach to receive food.

Gastric Digestion.—Digestion in the stomach includes chemical effects upon food and the mechanical reactions due to contractions of the stomach muscles. Certain aspects of the anatomy of the stomach must

be referred to. For this purpose, Fig. 132 serves to give a resume. Some of the muscle fibers of the stomach are arranged circularly. These fibers are especially developed to form the **prepyloric sphincter**, which by its constriction tends to divide the stomach into two chambers (1) the pyloric region and (2) the larger cavity which includes the fundus and the



FIG. 133.—Gastric glands. *A*, pyloric (index lines show the lumen of each gland). *B*, group of two fundic glands showing the numerous parietal cells. *A* $\times 100$, *B* $\times 150$. (After Lewis and Stöhr's "Text Book of Histology.")

cardiac region. The circular fibers also have a marked development at the pylorus. These fibers form the **pyloric sphincter** which by its contraction can close the opening from the stomach into the intestine.

The Gastric Glands.—The lining of the stomach (the gastric mucosa) is deeply pitted by very numerous glands. These are the source of the gastric juice. Each one has the form of a somewhat crooked tube with fine bore opening into the stomach cavity and thick walls composed of secreting cells. The individual gastric gland is comparable to a single

ascinus of a compound structure like a salivary gland. There are two different types of gastric glands. Those in the pyloric region, sometimes called **pyloric glands**, are made up of a single layer of secreting cells. (Fig. 133a.) Very rarely these glands have a few large cells lying outside of the main layer which forms the wall. These accessory structures are called **parietal cells**, or border cells. The other type of gland predominates in the main part of the stomach, the fundus. These are called **fundic glands**. They are characterized by large numbers of parietal cells, especially in the prepyloric region (Fig. 133b). During prolonged activity of gastric glands, the cells which compose the walls, (the chief cells) become noticeably shrunken as do active salivary cells, but the parietal cells do not greatly change in size or structure. Inasmuch as the mucosa of the prepyloric region is always distinctly acid when its surface is tested in the stomach of a recently killed animal, while the pyloric region is not acid, the fundic glands are believed to secrete most of the acid of the gastric juice. The parietal cells, characteristic of these glands, are therefore supposed to be concerned with the production of acid. Indeed, the acidity of any part of the gastric mucosa is roughly proportional to the relative abundance of parietal cells in the region. Cells of animal tissues are alkaline in general and the fluids of the animal body are also generally alkaline. The production of acid by the parietal cells is thus exceptional. The fact that this acid is the mineral acid, hydrochloric, and is produced in concentration sufficient to destroy most living cells, makes the phenomenon all the more remarkable. The gastric glands, like all other glands, are bathed in the alkaline lymph supplied by alkaline blood. The mechanism by which a secretion of comparatively high concentration of hydrogen ions is produced by cells nurtured in a medium of $\text{pH} = 7.35$ (average reaction of blood), can only be conjectured at present. The sodium chloride of the blood is doubtless utilized for this production of hydrochloric acid. This is indicated by the formation of hydriotic or hydrobromic acid in the gastric juice, after sodium iodide or sodium bromide is injected into the blood.

Methods for Obtaining Gastric Juice.—Gastric juice mixed with more or less food material, can be obtained by emptying out the contents of the stomach of a recently killed animal. This gives no adequate idea of the true composition of the gastric juice. Removal of gastric contents of a living animal or man by use of the stomach pump is also an unsatisfactory method for obtaining gastric juice. The most successful method employs the **gastric fistula**.

The first gastric fistula came about accidentally in the famous case of Alexis St. Martin. He was wounded by the accidental discharge of a shot-gun. The wound involved the front of the abdomen and the stomach. In the process of healing, the stomach wound became attached to the abdomen so that a permanent opening from the outside of the body into the stomach cavity was established after the wound was

healed. A fold in the mucous lining of the stomach lay in such a position that it formed a flap or valve inside of the fistula. This enabled the stomach to retain food. Recovery was complete and the patient was in good health. Dr. Beaumont, his attending physician, published in 1828, a unique account of this case and his numerous observations on it. The important physiological outcome was the development of an operative method for making the gastric fistula. There have been only a few cases of the gastric fistula operation on human beings. The one observed by Carlson, as mentioned above, was necessitated because of occlusion of the oesophagus.

For the most part, gastric fistulas have been made on dogs. Heidenhain largely developed the experimental technique, but the operation was particularly elaborated for the study of gastric secretion by Pawlow. He devised a method by which the stomach was divided into two parts. The main part of the stomach was left in a condition to carry on its functions as usual but a smaller part was made into an "accessory" stomach. This smaller sack was provided with a permanent fistula. The division of the stomach was made by a longitudinal cut running parallel to the main blood and nerve supplies of the stomach. In this way, the nutritive condition and the nervous control of both portions of the operated stomach were left in normal functional condition. While food was being digested in the main part of the stomach, normal gastric juice was produced also in the "accessory" stomach. This juice, uncontaminated by food material, could be collected at the fistula.

Artificial gastric juice, as it is usually called, is suitable for the experimental study of the action of stomach enzymes. This preparation is made by extracting the ground up mucus lining of the stomach of a recently killed animal. For this extraction, several different solvents are suitable: 0.4 per cent HCl, water, glycerol, mixtures of water and glycerol or mixtures of water and alcohol. The extract made with dilute HCl can be treated with an excess of alcohol so as to form a precipitate which contains the gastric enzymes. Dehydrated by further treatment with pure alcohol, this precipitate constitutes one of the forms of commercial pepsin used for medicinal purposes. A solution of this material in dilute HCl is an effective artificial gastric juice. Other forms of commercial pepsin are also produced by evaporation at 30 to 40°C. of neutralized extracts. Glycerol extracts of the gastric mucosa are dispensed under the name "glycerol of pepsin." They contain the stomach enzymes and, when mixed with dilute HCl, furnish a satisfactory artificial gastric juice. Dilute alcoholic extracts of the stomach glands are used as commercial "rennet" for culinary preparation of clotted milk.

The Composition of Gastric Juice.—Specimens of pure gastric juice, as obtained from the Pawlow "accessory" stomach, are clear, slightly viscid and, but for their pale straw color, have the appearance of saliva. The most striking feature is the acidity. The gastric juice contains hydrochloric acid in concentrations varying from about 0.2 to 0.5 per cent. The average of the supposedly most normal specimens from the dog is

0.4 per cent. Probably human gastric juice is nearly if not quite as acid as that of the dog. The gastric juice contains mucin-like proteins, inorganic salts and enzymes. The latter include **pepsin** (gastric protease), **rennin** (milk-coagulating, that is, casein-precipitating enzyme) and **gastric lipase**. The proportion of water to solids varies in gastric juice. Many specimens contain 3 per cent or more of solid matter; but the specimens regarded as most nearly normal for the dog, contain only about 0.5 per cent of solids and 99.5 per cent of water.

The Control of Gastric Secretion.—Secretion of gastric juice is excited reflexly as is that of saliva. An animal with a gastric fistula is seen to have gastric juice dripping from the fistula at the time that food is tasted, smelled or seen. The psychic or conditioned reflexes have been repeatedly shown to be effective in exciting gastric secretion. A hungry dog which has been regularly fed by the same attendant, will produce gastric juice on seeing this person. This occurs even when no food is in sight or where the dog could smell it. Further proof of the reflex control of gastric secretion is given by experiments in which Pawlow demonstrated that gastric juice can be abundantly secreted even when no food enters the stomach.

For this demonstration, he operated on dogs to produce a gastric fistula and in addition a double oesophageal fistula. The oesophagus was severed and the two ends were made to heal in to the skin of the neck. All food swallowed by the animal was thus prevented from passing to the stomach and was made to fall out of a hole in the neck. The animal was kept from starvation by collecting the material coming out of the oesophageal fistula and introducing it into the stomach through the lower fistula. Without this procedure, however, the animal could obtain only a "fictitious" meal as Pawlow named it. But during the time that the animal consumed the "fictitious" meal, a free flow of gastric juice was obtained. Since this work of Pawlow's, it has been repeatedly demonstrated that a human being, tasting, smelling or even thinking of food, is reflexly excited to secrete gastric juice, as is proved by pumping out the stomach after these stimuli are experienced.

The reflex center for gastric secretion is in the medulla. The efferent fibers reach the stomach by way of the vagus nerve. Electrical stimulation of the vagus nerve of an anaesthetized dog causes secretion of gastric juice. A dog in which both vagus nerves are cut does not secrete gastric juice during the eating of a "fictitious" meal.

Inhibition of gastric secretion undoubtedly occurs. Observations on human beings prove this. Persons under a strain of anxiety or other emotional states are often afflicted with indigestion which is due in part to a failure of gastric secretion. The nervous mechanism for this inhibition is probably a central one as in the case of the inhibition of salivary secretion. Nerves with a direct inhibitory effect have not been found. The splanchnic nerves can cause a partial, but indirect inhibitory effect upon gastric secretion by causing constriction of stomach arteries and thus decreasing the blood supply to gastric glands.

Although the reflex nervous excitation of gastric secretion controls the production of most of the gastric juice, another entirely different mechanism, a **chemical control**, is also effective. This mechanism depends upon the presence of certain food substances in the stomach. Not all foods can excite secretion of the gastric glands. A piece of bread, put into the stomach of a dog, through a fistula while the animal is asleep or at least unaware of the presence of food, excites no secretion and may even remain undigested in the stomach until putrefied by bacteria. Indeed, all the proteins, fats and carbohydrates, which occur in foods in their natural state, are powerless to exert a direct influence on the activity of gastric glands and can only arouse secretion when they are tasted or otherwise enabled to excite the reflex mechanism which controls gastric secretion. In contrast to the majority of food substances, the so-called **meat extractives** are effective local excitants of gastric secretion. These extractives are the substances which are dissolved from meat by boiling water and which give meat soups their flavor. The compounds included among the extractives are nitrogenous bases many of which are amino-compounds. A few of them are chemically related to the amino-acids. Several of them belong to the group of purine bases which are related to uric acid. Of the numerous compounds known to compose meat extractives, no particular ones have been shown to be the special excitants of gastric secretion. Soups and other meat concoctions or meats containing their extractives arouse the secretion of gastric juice when they are introduced into the stomach directly. A few other substances have a similar though less powerful effect. Among them are peptones, which were described above as products of the partial hydrolysis of proteins. All substances like meat extractives and peptones which excite secretion are called **secretagogues**. The mechanism of their action has been investigated. They do not act reflexly by exciting receptors in the stomach. This is proved by the fact that they effectively excite secretion after the vagus nerves are cut. But Edkins found that a water extract of the mucosa of the pyloric portion of the stomach of a recently killed animal, caused secretion of gastric juice in a dog. This was proved by injecting the extract subcutaneously or intravenously into a dog with a gastric fistula. Edkin's work has been repeatedly confirmed. If the extract of the pyloric part of the stomach is made with a solution of meat extractives or of peptones instead of with plain water, the resulting preparation is especially effective in exciting gastric secretion. Edkins suggested that the action of secretagogues is exerted upon the cells in the mucosa of the pyloric part of the stomach and there produces a hypothetical substance which he called **gastric secretin** or **gastrin**. The gastrin is supposed to be taken by the blood to all parts of the stomach mucosa and to specifically excite the gastric glands. Gastrin is one of the several internal secretions which go into the blood after they are

produced by special glands. The fact that such secretions are specific excitants of acitivity in other glands or in muscles led Bayliss and Starling to call them **hormones** (from the Greek, to arouse or call to action).

The normal operation of the mechanisms controlling gastric secretion, appears to be: (1) The reflex effects which occur in response to eating and which produce most of the gastric juice required to digest the meal; (2) the local chemical effect exerted by meat extractives after they reach the stomach; (3) a later excitation which occurs during the ordinary

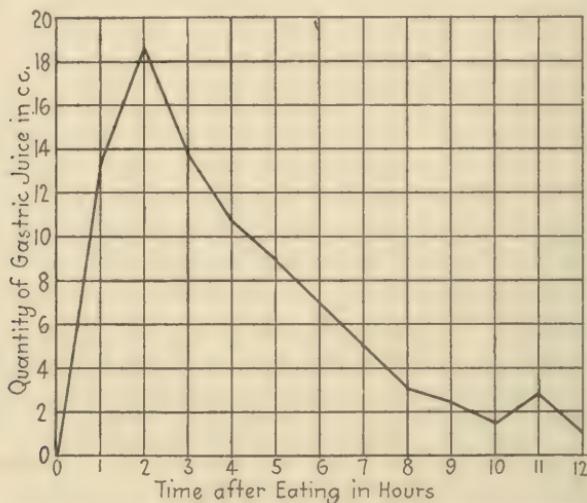


FIG. 134.—Curve to show the rate of secretion of gastric juice in the dog during digestion of a meal. The slight rise in the rate some 10 to 12 hours after eating is probably due to secretagogue action of the products of digestion. (After Khigine.)

course of digestion of a meal and is caused by the secretagogue action of the peptones produced by partial hydrolysis of food proteins in the stomach. The curve of the secretion of gastric juice, collected from a fistula in a dog and measured at hourly intervals following the eating of a meal (Fig. 134) shows that the reflex excitation produces a rapid rate of secretion which lasts during the first two or three hours. This nervous excitation is doubtless reinforced by chemical excitation which, as peptones are produced, causes a slight increase in the rate of secretion, some hours after eating.

Movements of the Stomach.—The best method for observation of normal muscular activity of the digestive organs is the X-ray method. As developed by Cannon, the procedure consisted of giving a suitable meal mixed with subnitrate of bismuth, an insoluble, harmless powder, which renders the food comparatively impervious to the X-rays so that a series of photographs of the stomach and intestines can be made to show the successive positions of these organs. This method has become familiar through its practical application in the diagnosis of digestive disturbances.

As applied to the stomach, this method shows movements that are characteristic. These movements are more easily described in connection with the arrangement of the fibers in the muscles of the stomach walls. These fibers are in two chief layers, the longitudinal, extending lengthwise of the stomach, and the circular, extending approximately at right angles to the longitudinal. A third type of fibers, the diagonal, also occurs but is less abundant. This arrangement of fibers enables the stomach to exert a sustained pressure upon its contents. The resulting slow contraction is the normal muscular activity of the cardiac portion and the fundus of the stomach. Thus the main part of the stomach merely presses upon the food and forces it toward the pyloric portion through the prepyloric sphincter. The tonus of the stomach muscles is constantly maintained in the manner characteristic of all non-striated muscles. The tonus in the normal stomach is always sufficient to keep its walls contracted to the extent permitted by the volume of its contents. The empty stomach, removed from an animal that was starved before death, is highly constricted. The active movements of the stomach are peristaltic waves of constriction. These start in the general region of the prepyloric sphincter and progress toward the pylorus. A single wave requires several minutes to traverse this distance and the separate waves may follow each other at intervals that are as short as 10 seconds. This motion gently pushes the contents of the pyloric region toward the intestine. If the contents are sufficiently acid, the pyloric sphincter relaxes long enough to permit some material to pass into the intestine. The amount leaving the stomach as the result of any one peristaltic wave is small because the pylorus does not long remain open. Its closure causes some of the stomach contents to move back through the central part of the pyloric region toward the fundus. In this way, the stomach contents are mixed and are very gradually worked out of the stomach into the intestine. This muscular activity of the stomach is subject to marked inhibitions. Emotional states, fear or rage, can entirely stop gastric peristalsis. Cannon showed this in experiments on laboratory animals. The stomach of the cat, for example, although working normally as shown by X-ray pictures at the beginning of an experiment, comes to a complete standstill when the animal is frightened or enraged by a threatening dog or by any other annoyance. The well-known effects of anxiety and rage in causing indigestion in human beings are due in part to the stoppage of gastric motility as well as to the interference with gastric secretion.

Innervation of the Stomach Muscles.—The walls of the stomach contain nerve plexuses. As these comprise ganglia as well as many branched nerve fibers they constitute a semi-independent outlying nervous system, in short, a nerve net. These plexuses account for the fact that the muscular activities of the stomach are orderly and nearly normal even

after all connections of the stomach with the central nervous system have been cut. Nevertheless, the stomach muscles are subject to regulation by impulses coming from the central nervous system. Certain fibers of the vagus are excitatory. This is shown by the fact that electrical stimuli applied to the vagus instigate marked muscular contractions or augment those already in progress. Excitation through the vagus can occur reflexly. This is shown by violent cramp-like contractions of the stomach which sometimes follow painful stimuli or emotional excitation. The stomach muscles are also involved in vomiting which is obviously a reflex act. To what extent the stomach muscles are normally influenced by reflex effects through the vagus, during undisturbed processes, is unknown. In addition to vagus fibers, the stomach has an extraneous nerve supply in the form of certain fibers coming from the splanchnic ganglia. Electrical stimulation of these fibers generally causes inhibition of muscular activity in the stomach. It has not been definitely shown that these splanchnic fibers inhibit the stomach muscle by a direct effect. Their excitation unfailingly causes vaso-constriction of the arteries of the stomach. The cessation of contractions of the stomach walls may be an indirect effect due to a decrease in blood supply. Inhibitions of gastric motility are, in any case, attributable to reflex effects which reach the stomach by way of autonomic fibers of the splanchnic system.

The Chemistry of Gastric Digestion.—The components of gastric juice which carry on the chemical changes of digestion in the stomach are hydrochloric acid and the enzymes, pepsin, rennin and lipase.

The Nature and Action of Pepsin.—The secreting cells of the gastric glands produce a forerunner or zymogen of pepsin. It is called **pepsinogen**. This substance is activated and becomes true pepsin by the effect of HCl. Presumably, the acid has little or no opportunity to come in contact with pepsinogen in the cells of the gastric glands. This appears to be due to the fact that whereas pepsinogen is produced by the chief cells of the glands, HCl is the product of the parietal cells. Activation probably occurs, then, in the lumen of the gastric glands or in the stomach cavity. The proof that pepsinogen rather than pepsin occurs in the glands is found in the following observation. An extract of chopped up gastric mucosa made with water or neutral salt solution or with glycerol is without enzyme effect. It becomes active only after it is brought to the proper degree of acidity. This latter fact alone does not prove the existence of a forerunner of pepsin because pepsin itself requires acid in order to show its activity. Indeed, pepsin that has once been acidified and is then neutralized, is inactive until it has been reacidified. The value of acid for peptic activity is really twofold. It activates pepsinogen and it furthers the work of the resulting pepsin. But further observations give a better demonstration of the distinction between pepsinogen and pepsin. When a water extract of the mucosa, that is, an extract of

pepsinogen, is made alkaline ($\text{pH} = 9$), is held at this reaction during about 30 minutes and is then brought to the acidity of normal gastric juice, active pepsin results. This proves that pepsinogen is not destroyed by such an exposure to alkalinity. But if an extract of the mucosa is first made acid and is then exposed to alkali in the manner just described, subsequent acidification does not restore peptic activity. In other words, pepsin, once really activated, is comparatively sensitive to the destructive effects of alkalinity.

The effects of pepsin cause the hydrolysis of proteins. The various stages of hydrolysis include: (1) Acid metaproteins, (2) proteoses and (3) peptones. These stages arise in the order mentioned, although all three of these types of hydrolysis products are present in varying proportions at any one time during the progress of digestion. This indicates that some of the protein is carried further in its hydrolytic cleavage than are other portions. If pepsin is permitted to act long enough on proteins, traces of amino-acids arise. Under ordinary circumstances, however, food proteins reach the stage at which a combination of different proteoses and peptones is present. The amino-acid stage is probably not reached even when some of the food remains in the stomach 12 hours or more.

Pepsin shows its maximum activity at an acidity represented by $\text{pH} = 1.7$. This is about equivalent to the acidity of $\frac{\text{N}}{50}$ HCl. The activity of pepsin is markedly diminished at $\text{pH} = 3$ and practically stopped at $\text{pH} = 4$. Acidities stronger than the optimum also check peptic activity and sufficient exposure to strong acid entirely destroys pepsin. If protein is previously soaked in hydrochloric acid until a protein—HCl combination is produced, pepsin readily hydrolyzes it in the presence of acidity lower than the optimum.

The Nature and Action of Rennin.—The milk coagulating enzyme, rennin, is also produced as a zymogen and is activated by hydrochloric acid. The proof of this is obtained by experiments analogous to those which differentiate pepsinogen and pepsin. Rennin is an enzyme which acts specifically upon the chief protein of milk. The transformation resulting from this action produces a protein of unique solubilities. The chief protein of milk is called, by some writers, casein and the corresponding product of the action of rennin is called paracasein. Other writers speak of the protein in milk as caseinogen and call the rennin-coagulated protein, casein. The latter convention will be adopted here. Caseinogen is held in solution in normal milk in the form of a calcium salt. From this combination with calcium, caseinogen is separated and rendered very insoluble if the milk is brought to the isoelectric point of caseinogen ($\text{pH} = 4.7$). This occurs when lactic acid is produced from milk sugar by bacterial action during the souring of milk. This results in clotting

of the milk because caseinogen, so precipitated, forms a semi-gelatinous mass. The same result can be obtained if milk is brought to this reaction by addition of any acid. The acid precipitation of caseinogen is reversible. Caseinogen redissolves in an alkaline solution and if the alkali used is calcium hydroxide, the soluble calcium salt of caseinogen is formed. But when caseinogen is acted on by rennin, the resulting precipitate is not formed by a reversible reaction. This reaction is entirely different from the acid precipitation. The casein, precipitated by the action of rennin, is thrown down in the form of a calcium salt and the reaction does not result in any precipitate or clot unless calcium ions are present in the mixture. Calcium ions are abundantly present in milk and combine with the casein as it is formed from caseinogen. If, however, calcium ions are removed from the milk by putting a sufficient amount of oxalates into it, the rennin clotting does not occur. Rennin produces casein even under these circumstances, as is shown by the fact that subsequent addition of calcium chloride causes the immediate precipitation of a calcium salt of casein. This reaction does not occur with caseinogen. It appears, then, that rennin transforms caseinogen into casein and the latter forms a clot because of its combination with calcium ions. The nature of the transforming action of rennin has not been definitely shown but it is probably a mild hydrolysis of caseinogen. This probability, together with the observation that rennin action is a property of the gastric juice of all animals, even of fishes and other animals which never consume milk, led to the theory, at one time defended, that milk coagulation is merely one aspect of the action of pepsin. But inasmuch as preparations showing peptic activity but without any milk coagulating power have been made, while other preparations which show rennin action but no general protein digesting powers have also been made, the separate identity of pepsin and rennin seems established. The function of rennin is to render milk suitable for gastric digestion. Without clotting, milk would pass into the intestine as soon as the gastric juice had acidified it. But in the clotted condition, milk is held in the stomach long enough to permit some action of pepsin and to relieve the intestine from the chance of undue crowding.

Gastric Lipase.—The fat splitting action of the gastric juice is not very marked. Finely emulsified fats, such as cream, may undergo a very partial hydrolysis into glycerol and fatty acids. But the acid condition of the stomach contents prevents any considerable change of this sort. Even when gastric juice is rendered alkaline, its fat splitting power is meager. Some experimenters have even contended that the gastric glands do not actually produce any lipase and that the mild fat splitting powers of the gastric contents are due entirely to a lipase which gets into the stomach by regurgitation of intestinal contents through the pylorus. Any marked lipase action is undoubtedly due to an enzyme

derived from this source. But the demonstration of a mild lipolytic action in extracts of the gastric mucosa renders highly probable the commonly accepted view that the gastric glands do produce a trace of lipase.

The Passage of Chyme through the Pylorus.—The pyloric sphincter is closed most of the time. The brief periods, during which the pylorus opens by relaxation of the sphincter, permit the stomach to pay out its contents slowly and bit by bit into the intestine. This prevents over-crowding of the intestine. The stomach is a storage place as well as a digestive organ. Its capacity makes an animal able to consume a large meal, as opportunity offers, instead of eating little and often. The action of the pyloric sphincter regulates the passage of gastric contents into the intestine. The material that passes through is called the **chyme**. It is generally described as having the consistency of a thick pea soup. Some suspended matter is present in it but is in finely divided condition. For the most part, food substances are in solution or in emulsion. The chyme is distinctly acid. This acidity is the chief stimulus for the requisite relaxation of the pyloric sphincter. Contact of acid with the mucosa on the gastric side of the pylorus causes inhibition of the sphincter. Consequently, peristaltic waves which carry along well-acidified gastric contents, tend to be followed by opening of the pylorus. This results, however, only when the gastric contents do not convey heavy solid masses to the pylorus. Such masses prevent the opening. Thus acidity and finely divided condition of the chyme are necessary for opening of the pylorus. After sufficient chyme has entered the intestine to bring acidity in contact with the intestinal lining, the pylorus closes. Both opening and closing are under regulation by a local nerve plexus. This nervous mechanism keeps the pylorus closed until acidity in the upper part of the intestine has been neutralized by alkaline juices secreted into it. The whole mechanism of pyloric action insures a regular and slow delivery of chyme into the intestine and prevents the passage of undigested stomach contents.

Intestinal Digestion.—At least three different juices are concerned in the digestive activity of the intestines of the higher vertebrates. Two of these, the pancreatic juice and the bile, are mixed with the chyme in the duodenum. The duct from the pancreas and the duct from the liver pour pancreatic juice and bile into the intestine through openings which are comparatively near the pylorus. The combined effects of these two juices cause the chief chemical changes of intestinal digestion. But intestinal digestion is also dependent, in a measure, upon the intestinal juice, generally called the *succus entericus*. This is formed by several types of glands which occur abundantly in the mucous lining of the intestine. The processes of secretion and the digestive activities of these three juices are conveniently described separately. It should be borne in mind, however, that they act conjointly.

The Pancreatic Juice.—Pancreatic juice can be obtained from a fistula. The operation for producing a pancreatic fistula is performed usually on a dog, through an incision in the abdominal wall. The point where the pancreas overlaps the intestine gives a rough indication of the location of the pancreatic duct. After exposure of the duct by careful dissection, a glass or metal canula is fastened into it. This canula is then so arranged that the other end of it protrudes from the incision. The dog recovers well from this operation and does not suffer any interference with normal digestion because in this species there are two well-developed pancreatic ducts, so that while pancreatic juice is drained off from one duct through the canula to the outside of the body, a fair supply of pancreatic juice flows through the other duct into the intestine. In rare instances, this operation has been necessitated in human patients because of cancers in the duodenal region. In such cases, however, the pancreatic fistula has been used as only a temporary expedient. But in the dog, the fistula can be made permanent and the incision wound permitted to heal around the canula.

Although the most accurate information concerning the composition and action of pancreatic juice has been obtained from the study of fistula specimens, another method is more frequently employed. This is the method of preparing *artificial pancreatic juice*. Extracts of fresh pancreas are made with water, dilute alcohol or glycerol and the resulting material brought to a faint alkalinity by the addition of dilute Na_2CO_3 . This solution possesses all the enzymic powers of true pancreatic juice. A modification of this method consists in desiccation of the fresh gland after its fat has been removed by absolute alcohol and ether. This desiccated material contains the pancreatic enzymes and may be kept indefinitely without deterioration of the enzymes. When required for use, some of the desiccated material is extracted with 0.5 per cent Na_2CO_3 . The resulting solution has the activities of pancreatic juice. The preparation of artificial pancreatic juice is still further modified as to some of the details of procedure but in any case the same principle, extraction of the gland and addition of suitable dilute alkali, is employed. Commercial products of this sort are dispensed under the names, *pancreatin*, *trypsin*, etc.

The composition of pancreatic juice from a fistula shows a high percentage of water, thus resembling saliva. The general appearance of pancreatic juice is also like that of saliva. The solids of this juice include proteins of several types, inorganic salts, among which, carbonates and phosphates are sufficient to render the juice alkaline, and several enzymes. The latter include: **Trypsin** (pancreatic protease), **amylopsin** (pancreatic amylase), **steapsin** (pancreatic lipase), **pancreatic rennin**, **lactase** and **maltase**. The reaction of pancreatic juice is more alkaline than that of any other animal fluids. Although varying somewhat, pancreatic juice usually has $\text{pH} = 9$.

The Chemistry of Pancreatic Digestion.—Constituents of the chyme undergo numerous and profound changes because of the effects of pancreatic enzymes. The diverse powers of the different enzymes furnished by the pancreas, enable its juice to hydrolyze all of the different kinds of typical animal foods. The bile, though lacking enzymes itself, furthers the activity of pancreatic juice. Pancreatic digestion in the small intestine breaks down food substances into the split products which are absorbed from the intestine and which are utilized for processes of animal metabolism. The pancreatic enzymes are thus indispensable for animals unless they are fed on predigested foods. The chemical effects of the saliva and the gastric juice are really only preliminary to the more important effects of pancreatic digestion.

The Nature and Action of Trypsin.—Trypsin, like pepsin, is secreted in the form of a zymogen. This is shown by the fact that fistula pancreatic juice, collected without any contamination, does not possess proteolytic powers. If, however, the pancreatic juice comes in contact with the lining of the intestine or if even a minute quantity of an extract of intestinal mucosa is added to the pancreatic juice, an active protease results. This activation occurs so readily that some difficulty is found in obtaining pancreatic juice free from protease. Probably, leucocytes of the blood and possibly some other cells may activate trypsinogen to form trypsin. The activation process shows all the characteristics of enzyme action, in that very minute amounts of intestinal material are sufficient to activate large quantities of pancreatic juice (catalytic effect) and intestinal material, that has been boiled, fails to activate. This appears, then, to be activation of one enzyme by another enzyme. The activating agent is therefore called **enterokinase**. This name refers to its normal production in the intestine and to its kinetic or activating powers.

The hydrolytic action of trypsin is exerted upon all proteins which are attacked by pepsin; but tryptic activity is more rapid and more complete when the proteins have been acted on previously by pepsin. The final products of tryptic proteolysis are amino-acids. All of the known amino-acids have been identified in intestinal contents and in mixtures of artificial or fistula pancreatic juice with food proteins. Amino-acids are not formed, however, in the first stages of tryptic proteolysis unless the proteins have been previously partially hydrolyzed as they are in gastric digestion. Trypsin acting on native, unhydrolyzed proteins causes them to pass through several intermediate stages of cleavage. The first stage is that of alkali metaprotein since trypsin acts in alkaline solution. Later stages include proteoses and peptones. If trypsin is permitted to act long enough, hydrolysis to amino-acids is complete in the case of most proteins.

The optimum reaction for tryptic activity is a distinctly alkaline one ($\text{pH} = 8$ to $\text{pH} = 9$). This alkalinity is attained in the intestinal con-

tents through the combined action of the alkaline salts in the pancreatic juice and those in the bile. They are sufficient not only to neutralize the acidity of the chyme but even to produce this degree of alkalinity.

Other Pancreatic Enzymes.—The amylopsin of pancreatic juice acts in the same manner as ptyalin of the saliva. It splits starches through the various stages of dextrins or acts upon the dextrins that have been previously produced by salivary digestion and forms maltose as the final product. The maltose is further split by maltase of the pancreatic juice and of the intestinal juice to form glucose (dextrose) as the final product of intestinal digestion of food polysaccharides. Similarly, lactase of the pancreatic juice and of the intestinal juice splits lactose (milk sugar) into glucose and galactose. The lipase of pancreatic juice hydrolyzes (saponifies) all food fats into their constituent fatty acids and glycerol. Inasmuch as this reaction occurs in the presence of the alkaline intestinal contents, the fatty acids combine with sodium and potassium ions to form soaps.

The Control of Pancreatic Secretion.—The stimulus for secretion of pancreatic juice is chiefly chemical. The earliest experiments, tending to demonstrate this fact, showed that as each small portion of chyme entered the intestine, pancreatic juice flowed from a pancreatic fistula. Attempts to find what constituent of the chyme produced this effect demonstrated that acidity was the controlling factor. Injection of hydrochloric acid into the lumen of the small intestine causes secretion of pancreatic juice as does the entrance of chyme from the stomach. Any acid touching the lining of the small intestine calls forth pancreatic secretion. These observations might possibly be taken to indicate reflex nervous excitation of the pancreas; but such is not the case, for when all the nervous connections of the pancreas are cut, the presence of acid in the duodenum produces a flow of pancreatic juice which is just as abundant as it is when the pancreatic nerves are intact. The real mechanism of pancreatic excitation was discovered by Bayliss and Starling. They showed that when an extract of the mucous lining of the duodenum of a recently killed animal is extracted with dilute hydrochloric acid, is neutralized, filtered and sterilized and is then injected into the circulation of a dog with a pancreatic fistula, pancreatic juice is secreted. This discovery was made before the work of Edkins on gastric secretin and was, indeed, the first discovery of typical chemical control of secretion. Bayliss and Starling suggested that a definite substance, produced in the intestinal epithelium by the action of acid, is carried by the blood to the pancreas and excites active secretion in pancreatic cells. They proposed to call this substance **pancreatic secretin** and suggested the term hormone to include all such chemical messengers. Numerous researches have been undertaken in the hope of proving the chemical identity of pancreatic secretin. These researches have shown the solubilities and precipitabilities of this hormone

and have shown that it is thermostable to the extent of resisting boiling temperatures and is diffusible through ordinary vegetable parchment paper. These observations are sufficient to prove that it is not a protein and, indeed, that it is not even a colloid. It is probably an organic compound of relatively simple structure but it has not yet been isolated, so that its chemical structure is still unknown. Secretin is not produced in parts of the intestine other than the duodenum. When the lining of the jejunum or the ileum is utilized for the same manipulations that are required for preparation of secretin, the resulting concoction, injected into an animal, does not excite pancreatic secretion. The possibility of some nervous excitation of pancreatic secretion cannot be entirely excluded. Electrical stimulation of the vagus nerve has been repeatedly observed to result in secretion of pancreatic juice. The amount of juice obtainable by this method is relatively small and the experiment does not always succeed owing to circumstances not yet fully understood. The blood supply to pancreatic tissues has some effect upon the secretory activity of the cells and in some cases active secretion in the pancreas is masked because of contractions of muscle fibers in the walls of the pancreatic duct. These contractions may temporarily close the duct so as to prevent the outflow of juice.

On the whole, the normal mechanism for regulation of pancreatic activity appears to be the following. Acid chyme enters the upper part of the small intestine. Its hydrochloric acid comes in contact with the surface of cells that form the intestinal lining. These cells then produce secretin and throw it off into the blood circulating through the walls of the intestine. The blood distributes it to all parts of the body but the pancreatic cells are specifically excited by it.

The Relation of Bile to Pancreatic Digestion.—The very considerable assistance which bile furnishes to pancreatic digestion can be shown in test tube experiments in which bile is added to artificial pancreatic juice. All the pancreatic enzymes act more effectively with bile than without it; but lipase is especially favored by the presence of bile. This is due, in large part, to the emulsifying powers of bile salts. As mentioned above, these substances are the most effective of the known emulsifiers of fat. The fine emulsification, which they produce, enables lipase to attack fats very effectively. The importance of bile for pancreatic digestion is further demonstrated by observations on human beings. When bile is prevented from reaching the intestine, as often happens in cases of jaundice, utilization of fat is strikingly diminished. Of the food fat eaten, under such circumstances, 60 to 90 per cent is recoverable in the feces, whereas under ordinary circumstances, 90 per cent or more of the food fat disappears—that is, is absorbed during intestinal digestion. Deficiency in the supply of bile prevents the digestion and therefore the utilization of fats. Inasmuch as fats tend to form a coating over parti-

cles of protein and carbohydrate in the chyme, they tend to prevent the access of water-soluble pancreatic enzymes into the interior of such food particles. On account of this, the failure of fat digestion during deficiency of bile causes a tendency to defective digestion of proteins and carbohydrates. It thus comes about that bile favors pancreatic digestion in general. It is possible that the presence of bile in the intestine has effects upon the process of absorption of products of digestion into the blood. Such effects are indicated by the fact that bile dissolves fatty acids and the otherwise insoluble calcium soaps. The dissolved condition of digestion products is probably requisite for their absorption. The distinct alkalinity of the bile is also useful, because it helps to neutralize the acidity of chyme.

The Composition of Bile.—Bile has a variable composition. The variations depend in part upon the condition of the liver and in part upon the length of time during which the bile is stored in the gall bladder. Bile can be collected as it flows directly from the liver without permitting its entrance into the gall bladder. This is accomplished by the operation of making a so-called biliary fistula, for which a canula is inserted into the bile duct. The liver bile, thus collected, usually contains over 97 per cent of water but the bladder bile, collected from the gall bladders of recently killed animals, is less watery and may contain as little as 82 per cent of water. Correspondingly, liver bile is more fluid, that is, less viscous than bladder bile. Liver bile is usually transparent while bladder bile usually contains abundant suspended matter. The explanation of the difference between these two kinds of bile is supposed to be the absorption of water from the bile through the walls of the gall bladder. It is also likely that some bile constituents are added by secretory action of cells in the lining of the gall bladder.

The chief solid constituents of bile are proteins, inorganic salts, bile pigments, bile salts, cholesterol, lecithin and other lipid constituents.

Their amounts are indicated in the following tabulation of representative analyses. No enzymes have been identified in the bile.

	Averages of 3 analyses of fistula bile from <i>liver</i> , per cent	Averages of 4 analyses of bile from <i>bladder</i> , per cent
Water.....	97.13	86.00
Bile salts.....	1.55	8.20
Mucin and pigments.....	0.49	2.25
Cholesterol.....	0.12	2.17
Fat, including lecithin.....	0.06	0.66
Inorganic salts.....	0.72	0.78

The Bile Salts.—Of these constituents, the bile salts are of prime physiological interest. They are chiefly the sodium salts of *glycocholic* and *taurocholic* acids. These salts can be crystallized in pure form by mixing ether with an alcohol extract of desiccated bile. Some of their properties were described above. They are soluble in water and alcohol and their solutions are distinctly alkaline. They yield precipitates of their free acids when their solutions are acidified with mineral acid. They have a remarkable power of lowering the surface tension of water. It is the latter property which enables them to emulsify fats and which confers emulsifying power upon the bile. This property is also due in part to the power of bile salt solutions to dissolve fatty acids and the water-insoluble soaps. This solvent power is much greater when the bile salts are accompanied by the lipoids of the bile and especially when in the presence of cholesterol. Glycocholic acid, when heated with dilute (5 per cent) hydrochloric acid, is hydrolyzed to yield the amino-acid **glycocoll** and **cholic acid**. Taurocholic acid, when similarly treated, also yields cholic acid but in addition yields **taurine**. The latter is related to the amino-acid, cystine, which upon reduction yields two molecules of taurine. Taurine is formed in the liver, presumably, from cystine. Cholic acid ($C_{24}H_{40}O_5$) is peculiarly the product of liver cells. Its formula has not been definitely established but its structure has been investigated sufficiently to show that it is a derivation of the group of terpenes. It is therefore related to cholesterol. Whether liver cells produce cholic acid from cholesterol or from some other source is unknown. Cholic acid confers upon the bile salts their peculiar physiological properties.

Cholesterol and other so called lipoid compounds were described in Chap. III. Cholesterol has been especially investigated in connection with bile chemistry because it is present in larger amounts in the bile than in other biological fluids. Its origin is, in some measure, cholesterol of the food; but in herbivorous animals, at least, it must be either from the plant sterols (p. 72) or from other unknown sources. It is probable, indeed, that all animals synthesize some cholesterol in the liver from precursors which have not yet been recognized. Cholesterol is secreted into the bile by the cells lining the gall bladder as well as by the process of secretion in the liver. This is proved by the disproportionately high content of cholesterol in bladder bile as compared with its amount in liver bile. Cholesterol becomes so concentrated in bladder bile that it frequently tends to crystallize in the gall bladder. Because of this, its crystallization is the usual immediate cause of gall stones. Solutions of bile salts have the power to dissolve cholesterol which is insoluble in all other watery media.

In addition to cholesterol, more typical lipoid substances, such as *lecithin*, are found in bile. They have been previously described. They

are accompanied by traces of soaps. No significance has been shown as yet for the presence of fat-like compounds in bile.

The pigments of bile give it a distinctive color. There are two chief varieties, **bilirubin** and **biliverdin**. The first is a reddish pigment, the other is green. Variations in the color of the bile from different species of animals or from the same individual, under differing circumstances, are due to the varying proportions of these two pigments. Human bile is commonly bright golden brown. The bile of most herbivorous animals is distinctly green. Ox bile, however, is usually brown. The color of different specimens of bile shows all shades of reddish browns and greenish browns. Bilirubin and biliverdin are closely related chemically. Bilirubin is converted into biliverdin by mild oxidation and the reverse change is obtained by reduction. Each of these pigments is able to combine with bases because of two carboxyl groups in their molecules. They are probably in the form of sodium salts in the bile. The free pigments can be prepared in pure state from gall stones in which they are usually abundant. The gall stone is first extracted with ether to remove cholesterol. Ether does not dissolve the pigments. The undissolved residue is then treated with chloroform which dissolves bilirubin but not biliverdin. From the chloroform solution, crystals of bilirubin are obtained by slowly evaporating the chloroform. The portion of the gall stone left after both ether and chloroform extraction, is treated with alcohol which dissolves biliverdin. It is obtained as an amorphous powder upon evaporating the alcohol.

The chemical structure of these pigments has not been definitely established. The empirical formulas are probably; bilirubin, $C_{33}H_{36}N_4O_6$; biliverdin, $C_{33}H_{38}N_4O_8$. The molecular structure of these pigments includes four pyrrol groups. The pyrrol group is found, as explained in Chap. V, in numerous plant pigments, including chlorophyll, and in the blood pigment, hemoglobin. The group also occurs in certain amino-acids. Its presence shows the close relationship between bile pigments and other physiologically important pigments. Its relationship to hemoglobin suggests its origin. Hemoglobin, a compound protein, will be described in detail in the next chapter. For the present, it is sufficient to call attention to the fact that upon hydrolysis, hemoglobin yields the pigment hematin. This substance contains four pyrrol groups. It is the forerunner of bile pigments. In old blood clots, that have been exposed to oxidation by the air and in bruises, "black and blue" spots, a pigment called hematoporphyrin is found. This is probably isomeric with bilirubin but is claimed by some investigators to be actually identical with it. Liver cells take up red corpuscles of the blood by the process of phagocytosis and cause their disintegration. This process involves the liberation of hematin and its oxidation to bilirubin. Further oxidation produces biliverdin. Still other pigments may be produced by oxidation

of biliverdin. Some of them have been found in bile. When bile or any mixture containing bile pigments is treated with nitric acid or some other oxidizing agent a succession of colors, green, then blue, then purple, and finally yellow is produced. These are due to formation of oxidation products of bile pigments. Reactions of this type are used to detect bile in urine, when, as in jaundice, the flow of bile into the intestine is so checked that it is absorbed into the blood and excreted in the urine.

Bile as a Secretion and an Excretion.—The composition of the bile illustrates the general physiological principle that secretion processes are in part excretory in function. Bile contains materials, namely bile salts, that are typical secretions in that they are specifically elaborated in a given kind of cells and are useful in a specific way. Some constituents of bile are manufactured in the liver cells but are of no further use to the body. The bile pigments are excreted in the feces either in the same form in which they are produced by the liver or in a somewhat modified form resulting from the reducing action of hydrogen and other gases produced by bacteria in the intestines. The bile pigments are a typical excretion. The case of cholesterol is somewhat similar. It is either removed from the blood by the liver or is produced in the liver and when conveyed by the bile to the intestines is excreted, in part at least, with the feces. Other constituents of the bile, for example, inorganic salts are not necessarily produced in the liver but are simply passed out from the blood through the liver cells into the bile. The excretory function of the liver in removing cholesterol and bile pigments is as indispensable to the body as is the production of bile salts to aid in digestion.

Control of Secretion of Bile.—The secretory activity of liver cells is more nearly continuous than is that of other digestive glands. This is shown by observations on the flow of bile from a fistula. The biliary fistula operation is necessitated, not uncommonly, in human beings because of disease of the gall bladder. From such fistulas, the rate of secretion of bile can be observed. The following table shows the average hourly secretion of bile in one specific observation lasting 48 hours.

	CUBIC CENTIMETERS PER HOUR
8 A.M. to 2 P.M.	25
2 P.M. to 8 P.M.	14
8 P.M. to 2 A.M.	16
2 A.M. to 8 A.M.	23
8 A.M. to 2 P.M.	28
2 P.M. to 8 P.M.	22
8 P.M. to 2 A.M.	12
2 A.M. to 8 A.M.	18

The rate of secretion increases during the middle of the day and declines at night. No reflex excitation of secretion in the liver cells has been demonstrated. In some cases, an increased flow of bile results from

certain experimental conditions involving nervous stimulation; but in every such case an increased flow of blood through the liver can be demonstrated. The general nutritive condition of the body and the abundance of the liver blood supply doubtless affect biliary secretion as they affect activities of all cells. In an indirect way, then, the nervous system can affect the activity of the liver through vasomotor control of its blood supply.

Although the secretion of bile is uninterrupted, the process is subject to chemical stimulation. The substances which act as hepatic secretagogues, that is, the **cholagogues**, are chiefly the bile salts. This is shown by the fact that the presence of bile in the intestine stimulates secretion in the liver. For this reason, bile is sometimes prescribed in jaundice and in other conditions which require stimulation of the liver to increased bile production. The same effects are produced by ingestion of purified bile salts, so that they are shown to be the active agents. The smallness of the amount of bile salts and their decomposition products normally present in the feces, indicates that bile salts are largely or entirely absorbed from the intestine. The inference is that bile salts effect a continuous circulation, coming into the intestine in the bile, being absorbed through the walls of the intestine, proceeding to the liver by way of the portal blood system, stimulating the liver to increased secretory activity and then being put out again with the bile into the intestine.

Not only does the presence of bile in the intestine stimulate bile secretion but also the presence of acid has the same effect. This recalls the chemical excitation of secretion of pancreatic juice under the influence of a hormone, pancreatic secretin. The intravenous injection of secretin preparations does, indeed, cause some increase in the rate of bile secretion at the same time that it causes a flow of pancreatic juice. This has been shown in experimental animals with a biliary fistula. As the active principle of secretin preparations has not been identified, there is no proof that the same substance which excites the pancreas also excites the liver; but we may at least conclude that contact of acid with the lining cells of the small intestine produces something which aids in maintaining the secretory activity of liver cells.

The Amount of Bile Produced.—The only data to show the amount of bile produced per day, are the results of measurement of the flow from a biliary fistula. But as the diversion of the bile from the intestine removes one of the chief excitants of secretory activity in the liver, these observations give figures which are undoubtedly too low to represent the normal bile production. Even under these conditions, however, amounts in excess of 500 cc. per day have been repeatedly collected from a fistula in a human being. The amount seldom falls below 350 cc. per day except during starvation when secretion is distinctly diminished. An average of numerous fistula observations has led to an estimate of 15 gm. of bile,

per kilogram of body weight, per day. The character of the diet noticeably influences the production of bile. A high protein diet tends to increase the secretion of bile. Proteins are known to be able to cause increase in the secretion of hydrochloric acid in the stomach. The effect of acid in arousing secretin production in the intestine offers a possible explanation of the stimulation of liver activity by protein feeding. Any circumstance which causes destruction of red blood corpuscles (hemolysis) in the blood stream, tends to increase bile production.

The Flow of Bile from the Gall Bladder.—A part of the bile, secreted by the liver, is forced back from the common bile duct through its side branch which leads into the gall bladder. This is due to constriction of muscle fibers which form a small sphincter of the common bile duct at the point where it opens into the intestine. So long as the sphincter is contracted, bile collects in the bladder. At each gush of chyme through the pylorus into the intestine, there is a corresponding flow of bile out of the gall bladder. The gall bladder has a thin layer of non-striated muscle fibers in its walls. These fibers maintain some tonus which is increased at the moment of ejection of bile. These contractions force the bile out under pressure which, though small, is sufficient to eject the bile into the intestine under normal circumstances. The control of bladder contractions is a nervous reflex. The stimulus is the presence of acid in the intestine. The smallness of the pressure developed in the bladder accounts for the fact that when gall stones are dislodged from their place of formation in the bladder and get into the common bile duct, the flow of bile is not always sufficient to force them out into the intestine so that a surgical operation for their removal becomes necessary.

Movements of the Intestine.—The methods for observation of muscular movements are similar to those used for similar studies of the stomach. The best method is that employing X-rays. The intestines can be photographed after a "bismuth meal" has had sufficient time to be distributed along the intestines. Their shadows can also be observed continuously by the use of a fluorescent screen. Observations on excised intestines have been described (p. 291). In the normal digestive processes, two distinct types of motions occur, the segmenting or dividing movements and peristalsis.

The "*dividing motion*" as described by Cannon consists of constrictions of portions of the circular muscles at about equidistant regions. In watching such movements with the X-rays, one sees a loop or strip of intestine in the focus of the rays divide up into sac-like compartments, separated by the several constrictions as indicated in Fig. 135a. These contractions last only a few seconds. As these muscles relax contractions of others occur in the regions between the previously active ones (Fig. 135b). These relax in their turn and the whole series of motions goes on rhythmically at a rate of 20 to 30 contractions per minute. The

intestinal contents are thus segmented again and again and completely mixed. The stimuli regulating these activities, are the changing pressures of the intestinal contents against the walls. Such rhythmic movements do not appear to involve any extrinsic nerves but are probably

regulated by the nerve net of the intestine. They occur in the excised intestine.

From time to time, these segmenting rhythmic movements are interrupted by **diastalsis**. This is Cannon's name for the *peristaltic wave* which moves down the intestine, preceded by inhibition, in the same way that peristalsis occurs in the oesophagus. This moves the food further along and as the peristaltic wave dies out, the segmenting movements begin again. Bayliss and Starling

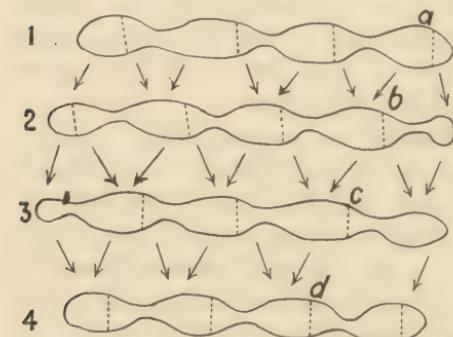


FIG. 135.—Diagram to indicate the dividing movements of the intestine. 1, 2, 3 and 4 show the form of the same portion of intestine at successive intervals. *a*, *b*, *c* and *d* show successive positions of a given particle. (After Cannon, *Am. J. Physiol.*, vol. 6.)

have described what they call the *law of the intestine*, namely that distention of the intestine at any point causes constriction of the muscles higher up (that is, toward the stomach) and relaxation of those lower down (that is, toward the anus). This reaction is also called the **myenteric reflex** as it involves the ganglionic plexuses of the intestinal nerve net and behaves like a localized reflex. Diastalsis occurs even when all nerve connections of the intestine are cut. The myenteric reflex depends upon the arrangement of the intestinal structures. This is shown by the results of experiments by Mall. He severed a portion of the intestine and reversed it so that its lower end was sutured to the upper part of the intestine and the previously upper end was sutured to the lower part. Some of the operated animals made a good recovery but later on showed serious nutritive disturbances. After they were killed, the intestinal contents were found to have accumulated just above the reversed piece of intestine which had evidently failed to propel food, although it appeared to be in functional condition save for its inverted position. The normal direction of the wave of peristalsis can be explained on the Von Uexküll principle of stretching of nerve nets (p. 341). In spite of its intrinsic and autonomous control, the intestine is subject to some influence of extrinsic nerves. Stimulation of vagus nerves augments intestinal contractions. Stimulation of the splanchnic nerves inhibits intestinal contractions.

A third type of intestinal movement occurs in the large intestine, particularly in the descending colon. It consists of a wave of contraction

which moves upward. It is sometimes named **anti-peristalsis**. But, as Cannon points out, it is not preceded by a wave of relaxation. He therefore proposes to distinguish it from peristalsis (diastalsis) by calling it **anastalsis** (ana-, upward). As a result of these waves, contents of the colon do not regularly pass into the rectum. When the anastalsis is replaced by downward motion material is propelled into the rectum and the stimulus for the reflex, voluntarily controlled act of defecation is thus given.

The Intestinal Juice.—In addition to the pancreatic juice and the bile, the juice secreted by the glands of the intestinal mucosa aids the digestive processes. This secretion can be collected from an intestinal fistula when the flow of intestinal contents from parts above the fistula is checked by a suitable plug. It has also been collected from isolated loops of the small intestine. There are no satisfactory estimates as to the amount of this juice produced. Its appearance and general characteristics are similar to those of saliva and pancreatic juice. The chief interest in it centers about its enzymes. These do not include characteristic proteases, amylases, or lipases although mild hydrolytic powers of these three types have sometimes been attributed to the intestinal juice. One of its most important enzymes is **enterokinase**. Its activity, in cooperation with pancreatic enzymes, was described above. The intestinal juice also contains sugar-splitting enzymes. **Sucrase**, **maltase** and **lactase** have been identified in the intestinal juices. These enzymes are not all found in the intestines of all animals though maltase appears to be universally present. The significance of these enzymes is seen from the fact that the disaccharides, sucrose, lactose and maltose, are not well utilized by the animal; so that their hydrolysis into monosaccharides, by the aid of these enzymes, is required to prepare them for use in metabolism. An enzyme called **erepsin** has been identified in the intestine. It causes the hydrolysis of proteoses and peptones into amino-acids. It does not act on the native proteins as do pepsin and trypsin. It therefore supplements the work of these two enzymes and insures that products of incomplete hydrolysis of proteins shall not be absorbed into the blood. This is significant in view of the facts of the individuality of animal proteins described in Chap. II. Another enzyme of the intestines is nuclease. This causes the partial hydrolysis of nucleic-acid derived from nucleoproteins.

Probably the five enzymes just described act inside of the cells which line the intestine. Their activity has been demonstrated, for the most part, in extracts of the intestinal mucosa. It is not definitely known to what extent they are actually secreted into the lumen of the intestine. It is possible that their chief, if not their only activity, is intracellular. Like these enzymes, the hormone, pancreatic secretin, may also fail to be actually put out into the intestine, but may be absorbed into the blood as soon as it is produced in the epithelial cells. It has been recognized only in extracts of the mucosa.

Absorption.—The products of digestion are not taken up into the circulation to any significant extent until they reach the small intestine. This is proved by experiments upon animals after the operation of making a duodenal fistula. In such animals, the chyme can be collected quantitatively and its composition compared with that of the food ingested. When this is done, the material recovered in the chyme is found to contain proteins slightly in excess of that eaten because of the additions of gastric juice to the stomach contents. The total carbohydrate and fat content is equivalent to that of the food. Only alcohol and a few other non-typical foods, such as pepper, mustard and other condiments, appear to be absorbed from the stomach. When they are absorbed, an accompanying absorption of ordinary food substances may also occur to a slight extent in the stomach. Inorganic salts are not absorbed unless they are present in the stomach contents in abnormally high concentration, 3 per cent or more. Water is not ordinarily absorbed in the stomach.

The small intestine is the chief absorbing organ. Its lining epithelium is adapted to this function. Its great length furnishes an extensive absorbing field. Its area is particularly large because of the enormous number of small projections, the *villi*, which occur in the lining of the small intestine. These projections afford an absorbing surface for the intestine even while a flow of intestinal juice is simultaneously coming out of the glands of the intestinal mucosa. The villi are adequately exposed to the liquid material in the intestine because intestinal contractions keep the food and its digestion products constantly in motion. Moreover, the villi possess some contractility of their own. Small muscle fibers, within the villi of some species are strong enough to retract these organs or to give them a slight waving motion. At least, this can be seen to occur on the exposed surface of a fresh preparation of intestinal mucosa when it is observed with low magnification.

Materials taken up from the small intestine can leave it by way of two different paths: (1) The path by way of the *blood vessels* includes the capillaries in the walls of the intestine and especially those in the villi, the mesenteric veins and the portal vein. Any absorbed material taking this path is bound to pass through the liver before it enters the general circulation of the body. (2) The path by way of the *lymphatics* includes the lymph spaces of the villi and other lymph vessels of the intestine, the large lacteal vessels and the thoracic duct. Materials taking this path reach the blood indirectly as the thoracic duct empties into the venous system near the heart. The material which leaves the intestine by way of the lacteals is called the *chyle*.

The absorption of water occurs in all parts of the intestine. The amount taken up in the small intestine is not sufficient, however, to cause any noticeable decrease in the fluidity of the intestinal contents. Material passing through the ileocecal valve into the large intestine has

about the same consistency as chyme. This is possible, in spite of absorption of water, because the secretions pouring into the intestine add to its contents sufficient water to compensate for that absorbed. Absorption of water in the large intestine is much more noticeable and is sufficient in most animals to cause the formation of semi-solid or even hard feces under ordinary circumstances.

The absorption of protein digestion products yields amino-acids to the blood. Although older views regarded the soluble and diffusible proteoses and peptones as suitable for absorption, the modern view is that even if they are absorbed they are so changed by erepsin in the intestinal walls that amino-acids are the only substances, resulting from protein digestion, that reach the blood. Analyses to determine the quantity of amino-acid in the blood at frequent intervals show a distinct increase during the digestion of a protein-rich meal. The amino-acids do not leave the intestine in detectable amounts by way of the lacteal system. This is proved in experiments on dogs, provided with a thoracic duct fistula. The lymph, collected from such a fistula, does not contain any increased amount of nitrogen-containing compounds as a result of protein digestion.

The absorption of carbohydrate digestion products yields monosaccharides to the blood. Glucose is the normal sugar of the blood in all animals. It is distinctly increased in the blood of the portal vein during digestion of carbohydrate food. Other monosaccharides are also absorbed, though quantitative measurements of the small amounts normally present in the blood are not entirely successful. The necessity of hydrolysis of colloidal polysaccharides to fit them for absorption is obvious, but the hydrolysis of the very soluble and diffusible disaccharides to monosaccharides is more surprising. The sugar-splitting enzymes, maltase, lactase and sucrase, of the small intestine tend to insure the conversion of all utilizable food carbohydrates into monosaccharides. As previously emphasized, the latter are more effectively used by animal cells than are other carbohydrates. Even the monosaccharides, other than glucose, are not entirely suitable. Levulose and galactose are known to be transformed by the liver into glucose. It is probable that no carbohydrate is oxidized in the normal metabolic processes of the animal until it has first been transformed into glucose. Sugars, other than monosaccharides, are absorbed if they are present in the intestine in sufficiently high concentration. If sucrose, for example, is eaten in large amounts, it will be detectable in the blood and in the urine. The portal blood conveys sugars away from the intestine. The lymphatic path conveys, at most, a very small proportion of the absorbed sugar.

The absorption of fat digestion products occurs in the form of soaps, fatty acids and glycerol. This fact has been established with some difficulty, because these substances are not conveyed away from the

intestine as such but in the form of neutral fat. Indeed, histological preparations of intestinal mucosa show an abundance of droplets of emulsified fat in the villi and even in the cells of their free surfaces, that is, in the absorbing cells. The picture as seen in the microscope leads to the seemingly obvious, but probably quite erroneous, conclusion that fats are absorbed in finely emulsified condition. But finely emulsified fats do not disappear from the intestine in the absence of pancreatic juice and bile. Moreover, histological investigation has not shown any fat in the outer border zones of the absorbing cells, the region actually in contact with intestinal contents. The theory, most generally accepted, is that fats are absorbed in a hydrolyzed condition but are immediately resynthesized to neutral fats in the absorbing cells, chiefly those of the villi. Soaps, such as those formed during intestinal digestion, are toxic when introduced directly into the circulation by means of intravenous injection and are not found in normal blood. The necessity for the presence of bile salts indicates that they are concerned with fat absorption. Their marked power to dissolve fatty acids even suggests that the latter make use of molecules of bile salts as vehicles upon which they ride across the limiting membrane of the absorbing cells. This suggestion is in accord with the established fact that bile salts are themselves absorbed and returned to the liver. The sodium and potassium soaps and glycerol are soluble in water, so that a special solvent is not required for their absorption.

The path, followed by the resynthesized, absorbed fat, is the lymphatic system. One has only to see the lacteal vessels of an animal, killed while digesting fat-containing food, to be struck by the general truth of this statement. These vessels are seen to be gorged with chyle which is so loaded with emulsified fat as to appear milky. Hence the name lacteals. Collections of the chyle from a fistula in the thoracic duct contain about 60 per cent of the fat, known to be ingested with the food. As shown above, over 90 per cent of the food fat is normally taken up from the intestine. What becomes of that which is not recovered in the chyle? This question has not received an adequate answer. The content of neutral fat in the portal blood does not increase during the absorption of fat-rich food. Either a considerable part of the fat is oxidized in the walls of the intestine during processes of digestion and absorption or fat is conveyed by the portal blood in a form not yet recognized, or fat is stored in ways not yet recognized. In regard to the last possibility, the behavior of the leucocytes has been investigated. Leucocytes are very numerous in the lymph vessels and spaces of the intestine, especially during digestion. They are known to take up large quantities of fat most of which is given off to the chyle. But the leucocytes may themselves convey a part of the fat away from the intestines and store it in lymphatic glands or other tissues.

The nature of the absorption process has not yet been adequately explained. Earlier workers attempted to explain absorption upon the basis of diffusion in accordance with the laws of osmotic pressure. Later work has clearly shown that so far as those laws are now known, they are inadequate to explain absorption. Different kinds of inorganic salts and other kinds of soluble and diffusible substances are taken up from the intestine at entirely different rates as measured by their disappearance from tied-off loops of the intestine. This is true even when the substances are present in the intestine in equimolar concentration so that they exert equal osmotic pressure. Moreover, some of the blood serum of an animal is absorbed in due time from a loop of its own intestine. Presumably, the serum has the same osmotic pressure as the cell contents and many of the serum constituents exert the same osmotic pressure as do the same constituents in the cells. The absorbing surfaces of the intestine are composed of living cells. The absorption process is therefore the same in character as the processes of the penetration of substances into cells in general. This matter has been discussed in previous chapters. When the whole question of the permeability of cell surfaces is known, the peculiarities of intestinal absorption will be explained.

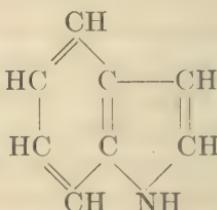
Bacterial Action in the Intestine.—Owing to the marked sterilizing action of hydrochloric acid of the gastric juice, the chyme as it enters the duodenum is normally in a nearly sterile condition. A few yeasts, some acid-producing bacteria and some bacterial spores are present in it; but putrefying organisms and most pathogenic forms are all, or nearly all, killed in the stomach. A mild antiseptic action is also attributed to the bile which does destroy many species of bacteria, though by no means all of them. The contents of the upper part of the small intestine do not comprise an abundant or diverse bacterial flora. In rare cases, they are found to be sterile. As material progresses further and further along the intestine, the bacterial content tends to increase steadily. Although certain types of bacteria usually predominate in the intestine of any given animal species, the flora of the intestine of any single individual animal is found to vary strikingly from time to time. Yeasts, fungi and other micro-organisms are not infrequently found. All these inhabitants of the intestine produce fermentative effects upon the food.

Carbohydrates are subjected to alcoholic fermentation and more extensively to acid fermentations. Because of the latter reaction, the intestinal contents are changed from the alkaline condition which prevails in the duodenum to an increasingly acid state as the material progresses through the lower part of the intestine.

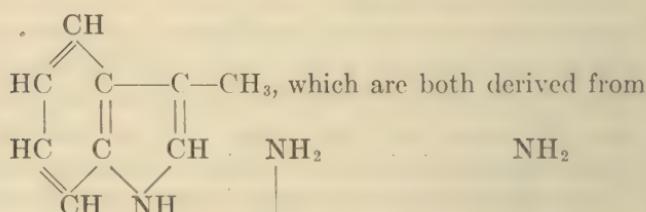
Proteins are subjected to putrefactive processes of which the products include organic acids and a long list of nitrogen-containing compounds commonly grouped together under the name ptomaines. This is perhaps something of a misnomer in that this name is used to apply to bacterial

and other products which are very toxic, while the products of intestinal putrefaction, though toxic, are normally not dangerous to the body because they can be transformed into harmless substances by the liver. A better name for these normal products of intestinal putrefactions is **aporrhegmas** (fragments) of amino-acids since they are all produced by a partial oxidative cleavage of the protein "building stones."

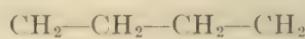
Among the best known ones are: **indol**, $\text{HC} \begin{array}{c} \diagup \\ \text{CH} \end{array} \text{C} \begin{array}{c} \diagdown \\ \text{HC} \end{array} \text{C} \begin{array}{c} \diagup \\ \text{CH} \end{array} \text{—CH}$ and **skatol**



(methyl indol), $\text{HC} \begin{array}{c} \diagup \\ \text{CH} \end{array} \text{C} \begin{array}{c} \diagdown \\ \text{HC} \end{array} \text{C} \begin{array}{c} \diagup \\ \text{CH} \end{array} \text{—CH}_3$, which are both derived from



tryptophane; **putrescine**,

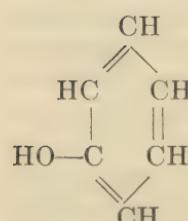
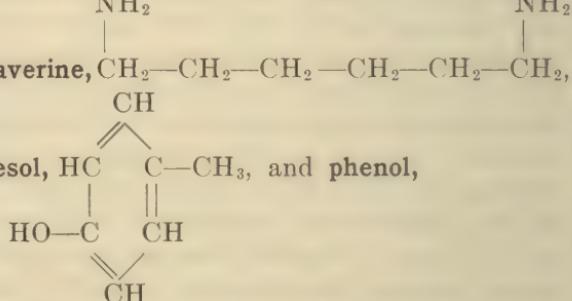


NH_2

derived from arginine; **cadaverine**, $\text{CH}_2 \text{—CH}_2 \text{—CH}_2 \text{—CH}_2 \text{—CH}_2 \text{—CH}_2$,

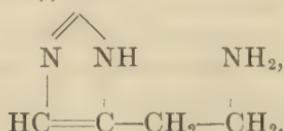
NH_2

derived from lysine; **p-cresol**, $\text{HC} \begin{array}{c} \diagup \\ \text{CH} \end{array} \text{C} \begin{array}{c} \diagdown \\ \text{HO} \end{array} \text{—CH}_3$, and **phenol**,



ethyl amine), CH

derived from histidine.



Most of these are amines which exert marked physiological effects upon blood pressure and other bodily conditions and are more or less toxic. The explanation of why their effects are not normally disastrous is found in the way the body disposes of them. They are absorbed into the portal blood which conveys them to the liver where they are either further oxidized or are combined with the sulphate group to form harmless compounds, the so-called **ethereal sulphates**. To what extent a failure of the liver to dispose completely of these aporrhegmas may be responsible for the well-known autointoxication, which often follows excessive intestinal putrefaction, is not definitely known. But inasmuch as many of the symptoms of autointoxication can be produced by injection of certain of these products into the general circulation, the inference is that the capacity of the liver to detoxicate them is limited and their excessive production results in their escape in toxic form into the general circulation.

Bacterial action causes the production of gases. The chief ones are: CO_2 , H_2S , CH_4 , N_2 and H_2 . The proportions of these gases vary with the character of the diet and of the intestinal flora. CO_2 and CH_4 are produced chiefly by fermentations of carbohydrates though they also result from other fermentations. H_2S and N_2 arise solely from putrefaction of proteins and their hydrolysis products. Ammonia is also formed but not as a gas for it combines with fermentation and other acids as fast as produced. Some of the nitrogen gas is derived from air swallowed with the food. Much of the gas, produced in the intestine, is not passed out through the rectum, but is absorbed into the blood. This permits the very toxic gas H_2S to gain access to the cells of the entire body.

To some extent bacterial action in the intestines is comparable, chemically, to the effects of the digestive enzymes. The intermediary products that arise during bacterial action include partially hydrolyzed proteins and amino-acids liberated from food proteins. Fats are hydrolyzed by bacterial action. The polysaccharides are split to their partial or complete hydrolysis products. For the most part, these bacterial processes are forestalled in the intestine by the powerful action of the enzymes of the digestive juices. But in one respect, the hydrolyzing powers of intestinal bacteria are significant for animal nutrition. Cellulose, a very large constituent of the food of herbivores, is broken down by the intestinal bacteria so as to yield soluble carbohydrate material whereas none of the digestive enzymes of the higher animals have any effect upon cellulose. Studies of the nutrition of herbivores prove that they do actually make use of cellulose. It would entirely escape digestion and utilization but for the bacterial flora of the digestive system. The organs of digestion in such animals, particularly in those with a large cecum, are arranged in such a way as to retain the food during a comparatively long time. This favors a more complete fermentation of cellulose than can occur in other animals. Aside from this utilization of cellulose, there is no satisfactory

evidence that intestinal bacteria are of use to the animal. Even herbivorous animals, guinea pigs, have been reared on sterile food, while kept under aseptic conditions after they were delivered by an aseptic Caesarian operation. In other words, they were properly nourished without any opportunity for bacterial infection of the intestines. The intestinal contents of polar bears are reported to be bacteria-free.

The Composition of Feces.—The feces contain indigestible materials, cellulose, etc. and undigested foods, such as starches, meat fibers, etc. The amount of these substances is commonly very small in human feces though cellulose constitutes a large proportion of the feces of some animals. To a considerable extent, the feces are composed of bacteria which comprise from *one-fourth* to *one-half* of the fecal matter. This is due to the rapid multiplication of bacteria in the large intestine. Very many of the fecal bacteria are dead owing to the accumulation of acids and other products of bacterial fermentation. Remains of the digestive secretions especially of the bile, are present in feces. As emphasized above, some of these are typical excretory products. The secretions formed by intestinal glands are also excretory to a limited extent. Calcium and some other inorganic constituents of the body are eliminated in this way. In the main, however, the feces represent the bacterial growth of the intestine and the unutilized residue of ingested materials rather than material that is, strictly speaking, excreted from the living parts of the animal.

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CHAPTER XVIII

THE CHEMISTRY OF THE BLOOD AND LYMPH

The blood is a circulating tissue. It is a tissue because it contains living cells. It is not a mere dead fluid but is capable of metabolic changes. Nevertheless, its chief function is that of conveying materials from one part of the body to another. The blood is frequently likened to the transportation system of a community. Just as a human population is unable to maintain its economic life without a transportation system for the distribution of the products of specialized industry, so the cell population of an animal body is unable to maintain its metabolic activities without a distributing system. Unicellular and smaller multicellular organisms do not require a circulating tissue because their cells are sufficiently exposed to the surrounding medium to be able to take their materials for metabolism directly from the environment and to give off the products of metabolism without difficulty. But the multicellular organism has the vast majority of its cells buried beyond any possibility of direct interchange of materials with the environment. Moreover, in the multicellular organisms, the specialization of cells to form organs makes them unfitted to carry on many of the functional activities that are necessary for their life. They thus become dependent upon the activities of other specialized cells. Muscles, nerves and glands are not capable of digesting foods to prepare them for metabolism. They are dependent upon the digestive system for this function and are also dependent upon the blood for the delivery of the products of digestion. Moreover, the products of many of the tissues of the body, the hormones, are required to control or affect processes in parts of the body distant from the producing organ. Inasmuch as all the nutrient materials and all the internal products of the different cells are to be found in the blood, a sufficiently complete description of the composition of the blood and its momentary changes would give an adequate picture of the entire process of metabolism in the animal. During recent years, the practical development of methods of blood analysis by the use of only small quantities of blood has made the studies of normal and abnormal metabolism available for diagnosis of disease.

The Structure of Blood.—Blood is composed of a fluid part, the *plasma*, in which are suspended the structural components, chiefly *corpuscles*. In human blood, plasma constitutes about two-thirds of the volume of the blood and the corpuscles one-third. The structural components

include the red corpuscles (erythrocytes), the white corpuscles (leucocytes and lymphocytes) and the blood platelets. In addition, the so-called blood dust is sometimes mentioned as being structural; but it probably consists of comparatively large colloidal particles suspended in the plasma. Such particles, like fat droplets in the blood, do not possess sufficient complexity of structure to make them comparable to cellular components like the corpuscles.

The red corpuscles vary considerably in different animals and are absent from the blood of most invertebrates. Among mammals, the biconcave disk form of non-nucleated erythrocyte is found in most species. Camels and other camelidae are exceptional in that they have red corpuscles which are flat, oval disks with a distinct nucleus. This latter form prevails in the amphibians and fishes. The size of erythrocytes varies greatly in different species. The average diameter of the red corpuscles varies in the same individual at different times. The average size for human beings in general is 7.7μ in diameter. Erythrocytes are very flexible and are often seen in a saucer or even a bell-shaped form. Their internal structure is unknown. The stroma envelope was described previously (p. 74).

The white corpuscles are full fledged cells, in that they are always nucleated. According to the different *forms* of their nuclei, the relative *size* of the entire cell, the cell *shape* (whether globular or irregularly amoeboid) and the *staining properties* of the protoplasm, leucocytes are elaborately classified. The proportion of the different classes present in the blood has been found to vary during diseased conditions, especially during infections, and has thus come to have a practical value in diagnosis of disease by blood examination. The typical leucocyte structure was described in Chap. X.

The blood platelets are much smaller than red or white corpuscles. They are round disks 1.5 to 3μ in diameter. They have not been found in the blood of birds, reptiles, amphibians and fishes. They are very difficult to observe in the blood of any animal, because they quickly disintegrate and entirely disappear when the blood is removed from the circulatory system. A special technique is required to preserve them for study. One method is to fix them with osmic acid. Their structure is not sufficiently complex to warrant ranking them as true living cells. They are described by some investigators as liquid crystals that is, a drop of solution having a definite structure. Their components include protein and lipoid substances.

The abundance of the structural components of the blood is subject to wide species and individual variations. The relative abundance of the corpuscles has been very extensively observed because of its practical application to diagnosis and treatment of disease and to the study of nutrition. The corpuscles can be counted by the apparatus shown in

Fig. 136. Under normal circumstances, the red corpuscles are from 500 to 1,000 times as numerous as white corpuscles. The average number of human red corpuscles is about 5,000,000 per cu. mm. Realizing that 1 cu. mm. is one fiftieth of an average-size drop, the total number of corpuscles in the 4 or 5 liters of blood in the adult man of average weight is seen to be very large. Leucocytes are said to be from 2,000 to 5,000 per

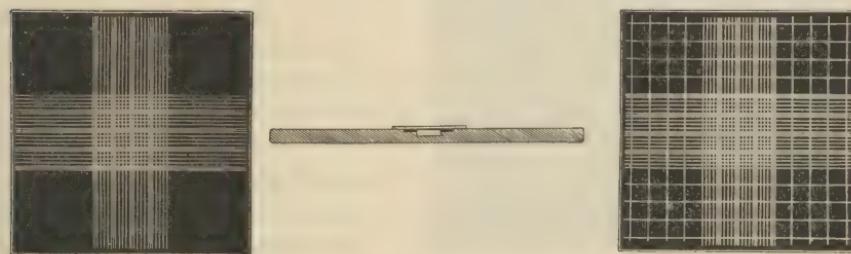


FIG. 136.—Blood-counting slide. A vertical section of the slide is shown in the center. The distance between the top of the counting platform and the bottom of the cover glass is 0.1 mm. Two forms of micrometer ruling for the counting area are shown, highly magnified, at left and right. The side of the smallest of the squares in each form is $\frac{1}{2}_0$ mm., so that the volume of the liquid seen in the field of the microscope over each square is $\frac{1}{2}_0 \times \frac{1}{2}_0 \times \frac{1}{1}_0 = \frac{1}{4}_000$ cu. mm. If blood is diluted 100 times before the corpuscles are counted, then the average number per square must be multiplied by 400,000 to obtain the number of corpuscles per cu. mm. of blood. The larger squares are used for counting leucocytes which, being less numerous than red corpuscles, must be enumerated in a larger volume in order to attain accuracy. (From the catalogue of A. H. Thomas Co.).

cu. mm., under normal conditions; but their number fluctuates very considerably. Enumerations of platelets in the blood have not been very extensively made. About 300,000 per cu. mm. is an average of some of the counts that have been made on human blood.

Coagulation.—The clotting or coagulation of blood occurs soon after it is drawn from the blood vessels. The usefulness of this property of the blood is the prevention of excessive hemorrhage from small wounds. The importance of this function becomes apparent in view of the plight of persons who lack it. These so-called "bleeders" are persons whose blood clots so slowly as to render them in danger of fatal hemorrhage. This condition is technically called **hemophilia**. It is hereditary.

The clotting of a large mass of blood, collected in a beaker results in a firm jelly which is completely set within a few minutes after the blood is drawn from the body. On standing about 24 hours, the clot contracts, draws away from the walls of the beaker, with the corpuscles enmeshed in the contracted jelly, and squeezes out a clear yellow or faintly red liquid called the serum. On close examination of the clot, it is found to consist of very fine microscopic threads of a substance called *fibrin*. Fibrin is a protein and separates from the blood during the clotting process in a felt work of interlacing needle crystals. Pictures of fibrin,

as the crystals are forming have been taken with the ultramicroscope. By this means, Howell has shown that fibrin formation in the blood involves crystallization. (Fig. 137.)

Fibrinogen.—The blood constituent, which is the chief component entering into the formation of fibrin, is a protein called fibrinogen. It



FIG. 137.—Fibrin crystals as seen with the ultramicroscope. (After Howell).

can be prepared from freshly drawn blood. The blood of the horse is sometimes used for this preparation because, if cooled immediately after it is taken from the veins, it does not clot very rapidly. By use of the centrifuge, the corpuscles are separated from the plasma. The plasma is then treated with an equal volume of saturated NaCl solution. This precipitates fibrinogen, somewhat contaminated by other blood plasma proteins. It is redissolved in a 3 per cent NaCl solution and again precipitated by a saturated NaCl solution. This method of dissolving and precipitating it identifies it with the group of proteins called globulins (p. 37). By a sufficient number of reprecipitations fibrinogen is obtainable in supposedly pure form. Dissolved in dilute salt solution, it does not clot spontaneously. It may be precipitated not only by salting out, but by any of the precipitation procedures used for ordinary proteins. But by any of these ordinary methods, the fibrinogen precipitate obtained is flocculent and amorphous. It is not crystalline. But if to a solution of purified fibrinogen a very small quantity of blood serum is added, the entire solution sets into a jelly due to the formation of fibrin crystals.

The production of fibrin is the immediate cause of clotting. This is shown by the failure of clotting in blood from which fibrin has been removed. This can be done by whipping or stirring the blood immediately after it is drawn from the animal, that is, during the time that clotting would take place if the blood were left undisturbed. The rod or stick used for this stirring becomes coated with a spongy mass of fibrin. All that forms in the blood can be collected in this way. The blood, from which fibrin has been removed, is called **defibrinated blood**. It can never clot.

The Need of Calcium Salts.—One of the necessary conditions for the transformation of fibrinogen into fibrin is the presence of soluble calcium salts, that is, calcium ions. If blood is collected by running it into a solution of an oxalate or if potassium oxalate is added to the blood as it is collected, no clotting occurs. The amount of oxalates necessary for

prevention of clotting is that known to be sufficient to precipitate all the calcium of the blood as calcium oxalate. Such "oxalated" blood clots immediately when calcium chloride or any other soluble calcium salt is added in excess of the combining power of the oxalates. The behavior of calcium in this reaction is closely parallel to its effect upon the clotting of milk by rennin.

Thrombin.—Fibrinogen and calcium salts are not sufficient alone to cause fibrin production. A substance present in the serum is required. It has been named thrombin, from the Greek word for a clot. It must be absent from normal blood while circulating, for otherwise the blood would clot within the body. The origin and nature of thrombin has been investigated with the hope of explaining the clotting process. Thrombin is best prepared by Howell's method. This consists in extracting thrombin and other material from water-washed blood fibrin with 8 per cent NaCl solution. This solution is then shaken with successive portions of chloroform, which removes much of the protein material in the extract. The latter eventually gets to a stage where boiling a test portion of it causes no precipitation of protein. This shows that all heat coagulable proteins have been removed. There still remains some protein in the extract. It has the properties of a proteose. It is very active in causing the clotting of fibrinogen solutions containing calcium ions. Although these preparations of thrombin are not known to be composed of a single purified substance yet they are so nearly purified as to justify the conclusion that the immediate cause of clotting is a reaction which may be represented thus:

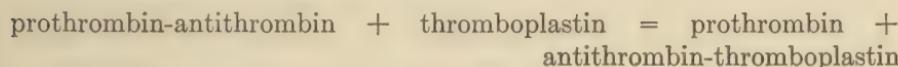


Older work indicated that thrombin might be an enzyme; but newer investigations have shown that for a given amount of thrombin a given quantity of fibrin is produced at least within certain limits. The enzyme character of thrombin is now defended by very few investigators. Whatever its nature may be, it is not in a condition to enter into the clotting reaction in the circulating blood. On this account, a hypothetical substance called **prothrombin** or **thrombogen** is assumed to be present in normal blood and to be changed into thrombin during the initial stages of the clotting process.

Thromboplastin.—Some substance, produced by dead or injured cells, must be concerned in this change. This is shown by the frequent observation that blood standing in a cut or wound clots much more quickly than it does when it flows out of an artery without coming in contact with injured tissues. Moreover, if the corpuscles and platelets of the blood are not subjected to disintegrating conditions, clotting is still more delayed or even prevented in shed blood. For example, blood flowing directly from an artery into a glass container lined with a coat of paraffin

and having paraffin oil floating upon the blood, will not clot for a very long time. Under these circumstances, the blood structures come in contact with nothing but paraffin, to which they do not adhere. They do not rapidly disintegrate as they do when they come in contact with glass and most solid bodies foreign to the blood. The inference is that normal clotting follows the disintegration of blood structures and the entrance into the blood of substances produced during the disintegration of any tissue cells. The clotting of blood is markedly hastened by the addition of extracts of most animal tissues. Indeed, this latter procedure is necessary to bring about the clotting of the blood of birds, reptiles, fishes and amphibians, if their bloods are collected directly from a blood vessel without any contact with wounded tissues. In these animals, blood platelets have not been found. This strongly suggests that, in the absence of products of injured tissue cells, disintegration of platelets is necessary for clotting. The disappearance of platelets from shed blood at the time it is clotting further suggests the same idea. Some observers claim that fibrin threads, as they form in a drop of fresh blood under the microscope, can be seen to appear first at the platelets and to radiate from them as starting points. It is, indeed, generally supposed that a substance liberated from the platelets and also from injured cells of most tissues, initiates the processes of clotting. The leucocytes of the blood have also been supposed by some writers to give off a substance that furthers clotting. But the evidence for this is not convincing. Wounded cells and blood platelets do yield something that contributes to the clot. It has been given various names, thrombokinase, cytozyme, etc. Howell has suggested the name, **thromboplastin** (clot former) or thromboplastic substance. This term has the advantage of suggesting the function of the substance without appearing to assume that it is an enzyme or that it has necessarily a given composition. Howell has been the most successful of those investigators who have attempted to show the nature of this substance. He has isolated and identified one compound at least, which has marked thromboplastic properties. This is the phospholipin, **cephalin**. This, as explained in Chap. III, is one of the nitrogen-containing phosphorized fats. It is closely allied to the lecithins. It had long been known that ether extracts of animal tissues contain something which markedly hastens blood clotting and when Howell discovered this effect of cephalin, the observations on ether extracts were explained, because cephalin is readily soluble in ether. The lecithins and all other known compounds, soluble in ether, have been shown to be without effect upon blood clotting. Although there may be substances other than cephalin which have thromboplastic action it is the chief and so far the only known thromboplastin soluble in ether. A thromboplastic substance present in blood serum is a **cephalin-protein compound**.

Two chief theories have been proposed to account for the rôle of thromboplastin in clotting. The older of these theories assumes that it activates prothrombin by changing it to thrombin. The newer theory, proposed by Howell, assumes that it reacts with some compound in the blood plasma so that prothrombin is liberated. The theory is based on the idea that blood is normally prevented from clotting within the circulation because prothrombin is in combination with some other substance, presumably another protein, called **antithrombin**. The reaction with thromboplastin is supposed to go thus:



The nature of antithrombin is unknown. Injection of Witte's peptone into the veins of an animal renders its blood incoagulable. Extracts of the heads of leeches also render blood incoagulable. This extract is called **hirudin**. It has been found useful in physiological experiments where the need of checking coagulation is met. Presumably all normal blood contains some antithrombic substance and a thromboplastin is required to counteract it before clotting can occur. Cephalin serves this purpose. It has been used practically in the form of emulsions placed upon bandages to hasten clotting and check the flow of blood from wounds. It also causes the coagulation of blood in hemophilia. This leads to the inference that hemophilia is due to the presence of abnormally large amounts of antithrombin in the blood.

Summary.—Several of the details of the clotting process are not yet clearly understood but the main facts are evident. They may be summarized as follows: Disintegrating platelets and wounded tissue cells give off thromboplastic substances such as cephalin and its compounds to the blood. This causes the production of thrombin or of prothrombin, but in the presence of calcium salts, thrombin is formed and reacts with fibrinogen to cause the production of a crystalline gel which is composed of interlacing fibrous crystals of the protein, fibrin, with other blood constituents held in the resulting meshes.

Intravascular Clotting.—Any circumstances causing destruction of blood platelets can produce clotting inside the blood vessels. Certain foreign substances if present in the blood will do this. For example, a needle protuded into a blood vessel is quickly surrounded by a clot. A bubble of gas lodging in a very small blood vessel so as to plug it, may in due time cause formation of a clot. Morbid conditions of cells in the walls of blood vessels may also produce clots. Thus the bruises produced in arteries during surgical operations may later cause the death of some of the cells in the injured area. A clot forms as a result. Any clot within the circulation of a living animal is called a **thrombosis**. If this clot is formed in a small artery or is moved by the circulation until it happens to

lodge in one, it will completely plug the artery, thus shutting off the blood supply to a neighboring locality. Without blood, tissues cannot live. If the plug lodges in the heart or in certain parts of the brain, the result is quickly fatal.

The Composition of Plasma.—The plasma contains a little over 80 per cent of water. Of the solid materials, *proteins* are the most abundant. They constitute from 6 to 8 per cent of the plasma, the proportion depending chiefly upon the species of the animal and to a much less extent upon individual variations. The proteins include fibrinogen, the serum albumins and the serum globulins. *Carbohydrate* material is not abundant in plasma. Glucose constitutes about 0.1 per cent of the plasma of most animals. Its amount fluctuates within narrow limits (0.06–0.15 per cent) under normal circumstances. It may be as high as 0.25 per cent under abnormal, pathological conditions. Sugars other than glucose may be present in the blood after they have been eaten in abnormally large quantities. Lactose may be thrown into the blood from the mammary gland when, during lactation, the milk is not removed from the gland. Unidentified carbohydrates of a polysaccharide nature are present in the blood in very minute traces. *True fats* are also very scarce in the blood plasma, constituting about 0.2 per cent. This amount is subject to some fluctuation but is probably never much higher under normal conditions and does not long remain higher under any conditions. Lipoids, cholesterol, cholesterol esters, lecithins and cephalin constitute about 0.3 per cent of the plasma. *Inorganic salts*, about 0.85 per cent of the plasma, include those found in all animal juices: The chlorides, carbonates, phosphates and sulphates of sodium, potassium, calcium and magnesium. The amounts of these salts are shown in the accompanying

INORGANIC CONSTITUENTS OF HUMAN PLASMA

	Per cent
Chlorides, as Cl.....	0.35
Sulphates, as H_2SO_4	0.013
Phosphates, as H_3PO_4	0.015
Potassium.....	0.031
Sodium.....	0.34
Calcium phosphate.....	0.03
Magnesium phosphate.....	0.022

table. The significance of the proportions of these salts to one another was discussed above. The amounts of all of them are subject to some fluctuations, within narrow limits. *Waste materials* thrown off from the different cells of the body include urea, uric acid, ammonium salts,

creatine, creatinine, purine bases, glycuronic acid and many other substances which, like these, are in the blood on their way to be excreted by the kidney or other organs of excretion. Dissolved *gases*, oxygen, carbon dioxide, nitrogen and gases from the intestine are present in the plasma. *Enzymes* found here include lipase, catalase and possibly some others. *Hormones*, such as the secretin described in Chap. XVII, and a number of others (see Chap. XXIV) can be detected in the plasma. *Immune substances*, such as antitoxins, are found in the plasma. An exhaustive list of all the substances that have been found in plasma or that are supposed to be there, at least under some circumstances, would be a list of nearly all substances concerned in the biochemistry of the higher animals.

Proteins of Plasma.—Fibrinogen was described above. Its amount has been estimated from the weight of fibrin which can be whipped out of the blood during defibrination. It amounts to about 0.5 per cent of the plasma. The serum albumins and globulins are very similar to the corresponding albumins and globulins obtained from eggs and other animal sources. Serum albumins are much richer in diamino-acids than are serum globulins. There are supposed to be several different albumins and globulins in the blood of each animal species. From the blood plasma, globulins are removed by varying degrees of saturation with inorganic salts. The albumin preparations from the same plasma coagulate at different temperatures. These slight differences in the properties of the globulins and the albumins found in any given specimen of plasma show that under artificial conditions in the test tube numerous proteins are present. There is evidence, however, that the manipulations required to separate proteins from plasma or serum cause some modification of the proteins, changing them from their natural form. Robertson has brought forward some evidence indicating that, in unaltered plasma, no globulin is present and that the proteins exist in some sort of a loose combination with one another—a protein complex. It is probably premature to say at present how many different proteins are actually present in circulating plasma. It is very probable that lipoids of the plasma are in some way combined with its proteins, contributing possibly to the formation of a protein complex. Chemical conditions in plasma are not as simple as they are in any artificial solution. Although plasma is generally regarded as a non-living solution, it is very close in its characteristics to a highly fluid protoplasm and is so regarded by some writers.

Composition of Red Corpuscles.—Roughly speaking, erythrocytes consist of a concentrated solution of hemoglobin in a stroma capsule. The stroma has been described (p. 75). Hemoglobin is the most abundant single constituent of the blood, constituting about 14 per cent of the entire blood in man and a somewhat smaller proportion in other animals. It constitutes about 32 per cent of the red corpuscles. Water composes a little over 60 per cent. Stroma constituents, lecithin, choles-

terol, inorganic salts and unidentified proteins, compose about 8 per cent.

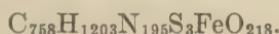
Hemolysis.—Any destruction of the form of red corpuscles is called hemolysis just as similar disintegrations of other cells are called cytolysis. Hemolysis by water and hypertonic salt solutions was described above in connection with phenomena of osmotic pressure. Other means of causing hemolysis are known. Shaking the blood with ether destroys the corpuscles by dissolving the lipoids of the stroma. Bile salts cause hemolysis, presumably because of their power to dissolve cholesterol. Mechanical effects, such as the pressure changes during alternate freezing and thawing of blood, can cause hemolysis. Snake venoms have a powerful hemolyzing effect. This is attributed to the presence of a hypothetical substance named **hemolysin**. Introduction of foreign blood into the circulation causes hemolysis, provided the introduced blood is taken from a not closely related species. The foreign blood is therefore said to contain hemolysin. Besides these natural hemolysins, **immune hemolysins** are known. For example, rabbit's blood introduced into a guinea pig, although causing no hemolysis in this related species does cause the development of hemolysin specific for rabbit's blood corpuscles; so that after repeated injections of rabbit's blood, the guinea pig has in its blood something which is hemolytic for rabbit's blood. Inasmuch as all immune substances are either proteins or involve the presence of proteins, hemolysins are supposed to be of a protein character, although their definite composition is unknown.

Hemolyzed blood takes on an appearance distinctly different from that of normal blood. It becomes transparent and of a deep, clear red color because it no longer refracts light from the surfaces of corpuscles. Because of this clear transparent color, hemolyzed blood is said to be laked or lakey blood.

Hemoglobin.—Hemoglobin readily combines with oxygen to form **oxyhemoglobin** which has the bright red color characteristic of arterial blood. This oxidative reaction is easily reversible; so that oxy-hemoglobin readily reduces to hemoglobin, giving off oxygen. This reduction occurs in response to any diminution of the tension (concentration) of oxygen in a hemoglobin solution or in blood. It is only necessary to expose blood to a vacuum or an oxygen-free atmosphere to entirely reduce oxyhemoglobin. Any sufficiently powerful reducing substance can cause the same result. Oxyhemoglobin is commonly reduced in the laboratory by ammonium sulphide or ammonium ferrous tartrate. These reagents do not otherwise alter hemoglobin. When so reduced, hemoglobin has the dark purplish or bluish color characteristic of venous blood. The reduction of oxyhemoglobin to hemoglobin, during the passage of blood corpuscles through the general tissues of the body, and the oxidation of hemoglobin to oxyhemoglobin, during their passage through the lungs,

gills or (in some animals) the skin, constitute essential processes of respiration which will be described in Chap. XX. The physiological value of this reversible action between hemoglobin and oxygen is seen in the resulting increase in the oxygen transporting power of the blood. Its capacity for absorbing oxygen from air is nearly seventy times as great as that of plasma alone. Plasma can carry oxygen only by dissolving it. Erythrocytes are the oxygen carriers of the blood and become so by virtue of their hemoglobin. It is therefore called a respiratory pigment. Without it blood plasma could not transport oxygen in quantity sufficient to maintain life of tissues. Hemoglobin is not the only so-called respiratory pigment. In certain invertebrates, **hemocyanin**, a copper-containing compound protein is found. Its behavior resembles that of hemoglobin (p. 566). Hemocyanin is bright blue in the oxidized condition and nearly colorless when reduced.

Hemoglobin is a compound protein. During mild hydrolysis with dilute mineral acid, it splits into a protein called **globin**, one of the histones, and a substance called **hematin**. The latter is soluble in alcohol which does not dissolve the globin; so that hematin can readily be separated and purified. It is an iron-containing compound. Its formula is probably $C_{34}H_{34}N_4FeO_5$. Its exact molecular structure is not established, but the four nitrogen atoms are known to stand for four pyrrol groups in the molecule. This shows its relationship to many plant pigments and to the bile pigments. Hematin is produced, in reality, by the hydrolysis of *oxy*hemoglobin because oxygen is not excluded during the reactions involved in its preparation. Like oxyhemoglobin, hematin itself can be made to give up oxygen to reducing agents. The resulting reduced compound is called **hemochromogen** or **reduced hematin**. This reaction is readily reversible. As the relations between hematin and hemochromogen are very similar to those between oxyhemoglobin and hemoglobin, the iron atom is assumed to confer upon hemoglobin its power to effect the remarkable and useful reaction with oxygen. On the assumption of the presence of one iron atom in the molecule of hemoglobin and the percentage of iron in it (0.335 per cent), the molecular weight of hemoglobin is given as 16,669. On the basis of this molecular weight and the determinations of its constituent elements, an empirical formula of hemoglobin is given as:

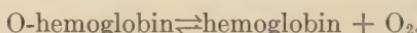


Combinations with gases other than oxygen can be made by hemoglobin. The combination with carbon monoxide (CO), called carboxy hemoglobin, has received special attention. Carbon monoxide combines, apparently, with the same valence of hemoglobin to which oxygen attaches. Because of this and because carboxyhemoglobin is a distinctly more stable compound than is oxyhemoglobin, carbon monoxide prevents

the union of oxygen with hemoglobin. This destroys a large part of the oxygen-carrying power of the blood. Carbon monoxide is therefore a deadly poisonous gas. It is the dangerous constituent of ordinary illuminating gas and of the waste gases from motors and smoldering coal fires. A person overpowered by nearly complete asphyxiation with a CO-containing gas is not apt to revive when brought out into fresh air, because even with a plentiful supply of oxygen, his blood is unable to take it up and transport it to the tissues. Carboxyhemoglobin does slowly dissociate, giving off CO through the lungs to air. If, therefore, artificial respiration can be maintained successfully, the oxygen-carrying power of the blood may be regained after a number of hours. In many cases it is necessary for resuscitation to resort to bleeding so as to remove some of the carboxyhemoglobin from the circulation. As hemorrhage stimulates the production of new red corpuscles, containing normal hemoglobin, the fatal effect of CO poisoning is sometimes prevented by this means.

The combination of hemoglobin with nitric oxide (NO) is entirely analogous to carboxyhemoglobin. Because of the nature of this combination, nitric oxide is poisonous.

The reaction of hemoglobin with CO₂ is very different from those just described. It does not consist of a union of CO₂ with the valence that ordinarily combines with oxygen. Nevertheless, the presence of CO₂ in blood or hemoglobin solutions does influence the dissociation of oxyhemoglobin. CO₂ disturbs the equilibrium of the reversible reaction:



The reaction is forced from left to right, that is, reduction is favored by CO₂. The same effect is also caused by increase in hydrogen ion concentration produced by any acid. The effect of CO₂ upon this reaction may be due solely to changes in C_H and does not signify that CO₂ combines with hemoglobin. CO₂, forming carbonic acid in solution, probably reacts with hemoglobin in the manner that any acid tends to react with proteins. It will combine with the protein on the more acid side of the isoelectric point (p. 247) but will not combine with it otherwise. The isoelectric point of oxyhemoglobin, according to Campbell and Poulton, is at pH = 6.98. An acid reaction would therefore seem to be required to permit an actual union between carbonic acid and hemoglobin. But acidity does not occur in the blood. Under natural conditions in the body, then, CO₂ probably forms no combination with hemoglobin.

The significance of the effect of CO₂ upon reduction of hemoglobin is that it facilitates a giving off of oxygen from hemoglobin to the body cells as the red corpuscles move through capillaries. The faster CO₂ is produced by oxidation in active tissues the faster does it tend to cause the liberation of oxygen for further oxidative reactions.

Absorption Spectra.—Like chlorophyll and other natural pigments, hemoglobin absorbs definite wave lengths of light. In other words, it gives an absorption spectrum. This is due to the hematin part of the molecule. Globin, like all colorless proteins gives no absorption spectrum. Very slight changes in the molecular arrangement of hemoglobin result in marked differences in the absorption spectrum. For example, the removal of one atom of oxygen in reduction of oxyhemoglobin to hemoglobin entirely changes the spectrum. Spectra obtained with hemoglobin and some of its derivations are shown in Fig. 138. The hemoglobin spectrum is characterized by a broad absorption band in the yellow green

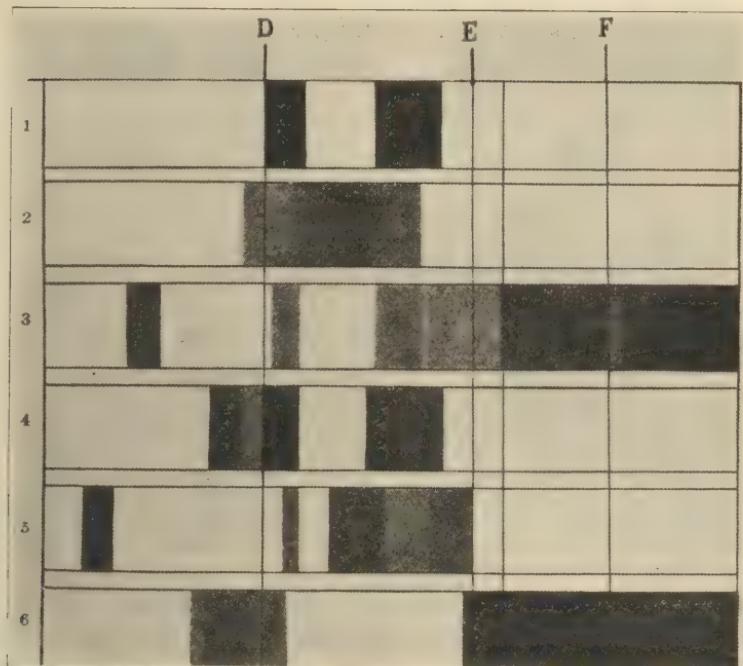


FIG. 138.—Absorption spectra of hemoglobin and its derivatives. 1, oxyhemoglobin. 2, hemoglobin in the presence of a reducing agent. 3, methemoglobin in neutral solution. 4, methemoglobin in alkaline solution. 5, hematin in acid solution. 6, hematin in alkaline solution. Fraunhofer lines are shown as points of reference. (After Ziemke and Müller).

region; that of oxyhemoglobin is characterized by two absorption bands, one in the orange-yellow region and one in the green region; the spectrum of methemoglobin which results from strongly alkaline oxidations of hemoglobin and from the action of aniline and certain other poisons, has several absorption bands of which the most characteristic one is that in the red region; the spectrum of CO-hemoglobin closely resembles that of hemoglobin but has its two characteristic bands shifted toward the violet end of the spectrum.

Under given conditions of observations, the light waves absorbed by hemoglobin or any one of its derivatives, are of certain definite lengths. As the latter can be accurately determined with the spectroscope, this instrument becomes of great practical use in quick and unequivocal detection of blood. The width of the absorption bands varies directly with the concentration of the hemoglobin or derivative in the solution observed. The spectroscope thus affords a method for rough quantitative estimations.

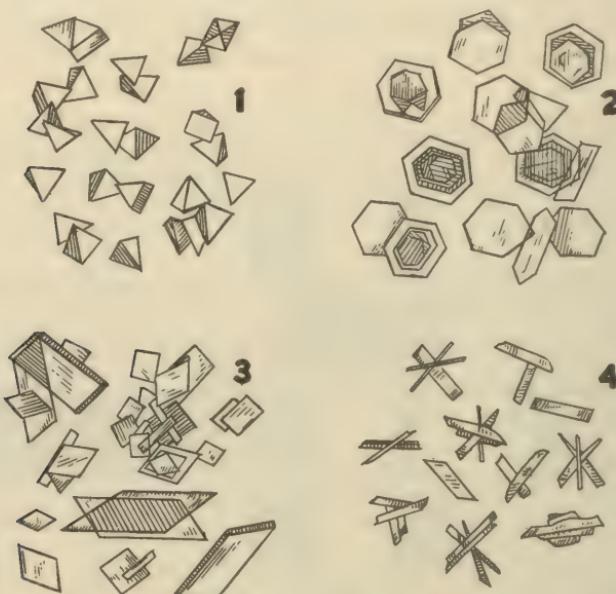


FIG. 139.—Sketches to indicate the forms of hemoglobin crystals as obtained from blood of different species. 1, guinea pig. 2, squirrel. 3, horse. 4, rat. (After Reichert).

tive estimations. By its aid, the diagnosis of mild gas poisoning or of poisoning which produces methemoglobin can be made with a small sample of blood.

Crystallization.—Hemoglobin readily crystallizes. One method for producing its crystals is to take the blood by shaking it with ether and let it stand for crystallization. This method is not successful with the blood of all species. Special conditions are required to induce crystallization in some kinds of blood. The crystals are distinctly different for the blood of each animal species. A few typical crystals are shown in Fig. 139. Reichert and his co-workers have made a careful crystallographic study to compare the differences between hemoglobin crystals with species relationships. They have obtained some remarkable results showing that not only do these crystals vary according to species but even according to genus. Moreover, those animals which are closely related according to taxonomy yield hemoglobin crystals which belong in the same crystallo-

graphic system. They have thus obtained chemical evidence which agrees with and confirms morphological classifications. The natural but probably unfounded inference from a study of these crystals is that there are as many different kinds of hemoglobin as there are species of animals with red blood. Doubt is cast upon this inference by several kinds of evidence. The proportion of iron in hemoglobin from widely different species is the same, according to some careful analyses. The chemical behavior and elementary analyses of hematin are always the same irrespective of the kind of blood from which it is prepared. This latter fact indicates that if there are species differences in hemoglobin, they must be due to the globin part of the molecule; yet globin prepared from one species does not behave like a foreign protein when injected into the blood of another species. It does not arouse immune reactions as do other proteins from foreign sources. There is some evidence to indicate that the crystal shape of hemoglobin is determined by the conditions under which it crystallizes, especially by the presence of other blood proteins. The serum proteins, albumins and globulins, are probably present in minute traces in hemoglobin crystals. These proteins are known to be absolutely specific for each animal species.

Hemin.—The action of nascent HCl upon hemoglobin and upon most of its iron-containing derivatives produces a compound called hemin. It is a combination of hematin with HCl and is therefore a hydrochloride ester of hematin. It readily crystallizes in dark chocolate-colored forms of definite and characteristic shape. (Fig. 140.) They are often called Teichmann's crystals. They may be either macroscopic or microscopic in size. It is possible to obtain them with very small quantities of blood even when it is old and partially decomposed. The material is placed on a microscope slide, treated with NaCl and concentrated acetic acid and is gently heated. After cooling, crystals may be found by a microscopic examination. As hematin does not occur in nature save as a part of the hemoglobin molecule, the value of this test for identification of blood is apparent. It has been extensively used in legal and medical practice. This test does not show the species from which the blood was obtained. The only way to show this is to employ biological tests in which the immune reactions of an animal body furnish the sensitive distinctions between the different kinds of proteins characteristic of species.

Leucocytes.—The term leucocytes (white corpuscles) includes numerous kinds of cells of diverse morphology. There are several classifications of the white corpuscles of human blood. In all of them, two main classes are distinguished: Lymphocytes and true leucocytes. The follow-

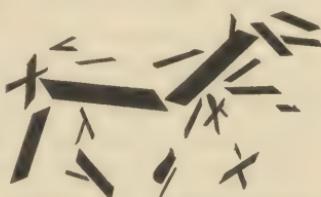


FIG. 140.—Hemin crystals.

ing classification of human white corpuscles is based upon that of Ehrlich and includes the features most generally agreed upon.

1. *Lymphocytes*, roughly spherical in shape, though differing in size; cytoplasm has only a few or very small and faint granules; single nucleus; not characteristically motile, though capable of change of form.

(a) *Small lymphocytes*, large centrally located nucleus; comparatively little cytoplasm; size about equal to that of red corpuscles (6 to 7μ in diameter); compose 22 to 25 per cent of the white corpuscles.

2. *True leucocytes* or *granulocytes* show amoeboid motion; have a distinctly granular cytoplasm; vary in size (8 to 10μ in diameter), always larger than human red corpuscles.

(a) *Polymorphonuclear leucocytes*, nucleus segmented into lobules connected by thin strands; most actively amoeboid of all white corpuscles; especially abundant, composing about 70 per cent of the white corpuscles; 62 per cent is regarded as the normal average, according to some investigators; subdivided into two groups: *Neutrophils*, of which granules stain slightly with neutral dyes or with combinations of acid and basic dyes, and *eosinophils* or *acidophils* of which the granules stain with eosin and other acid dyes. *Eosinophils* are not very numerous, 2 to 4 per cent of the white corpuscles.

(b) *Mast cells* or *basophils*, cytoplasm has coarse granules which stain with basic dyes, nucleus like that of (a); compose less than 1 per cent of the white corpuscles.

Monocytes formerly called *transition forms*, single large nucleus somewhat constricted into lobules; indistinct cytoplasmic granules which stain with neutral dyes; formerly supposed to be large lymphocytes that are in process of changing into polymorphonuclear leucocytes, but this supposition is now regarded as unfounded; compose 2 to 10 per cent of the white corpuscles.

The typical appearance of these different forms is shown in Fig. 141. Their recognition has assumed considerable practical significance because the relative abundance of the several types changes during diseased



FIG. 141.—Leucocytes. 1, lymphocyte. 2, large mononuclear-leucocyte. 3, eosinophils. 4, polymorphonuclear leucocytes. 5, leucocyte stained to show coarse granules. (After Lewis and Stöhr's "Text Book of Histology").

conditions. A differential count of the white corpuscles in a stained smear of blood on a microscope slide often gives what may be called "the blood picture" of a disease. The staining methods used and the results and interpretations will be found in laboratory manuals of diagnostic methods.

A marked increase in the total number of leucocytes of the blood is called **leucocytosis**. Although the normal number in a healthy person shows considerable variation, increasing somewhat after a large meal or vigorous exercise or exposure to cold weather or cold baths and varying during pregnancy, yet all these variations are usually within the limits 4,000 to 9,000 per cu.mm. It is therefore considered that 10,000 or more per cu. mm. indicates a pathological leucocytosis. It results from any general infection and may follow a localized infection.

The functions of the white corpuscles are supposed to be different for the different forms. The polymorphonuclear cells perform **phagocytosis**. This consists in the engulfing of foreign particles, especially bacteria, by means of amoeboid motion. Metchnikoff was the leader in the discovery and study of this process. The protection of the body from infecting bacteria by means of this phagocytic action is favored by the tendency of these leucocytes to collect in large numbers in any inflamed, that is, infected region. Leucocytes are very abundant in the pus forming in such locations. They escape from the blood stream into the infected region through the walls of blood capillaries. The wall is not itself ruptured or injured as these cells pass through. They apparently flow by amoeboid motion through interstices which they find or temporarily produce between the cells which compose the thin mosaic of capillary walls. The process by which leucocytes thus escape from the blood vessels is called **diapedesis**. The average number of bacteria engulfed by leucocytes varies with the chemical composition of the blood. Something present in the plasma and lymph especially favors phagocytosis. Corpuscles washed in physiological salt solution until they are free from plasma constituents fail to engulf many bacteria unless the latter have been treated with plasma, serum or lymph or whole blood. The hypothetical substance that favors phagocytosis is called an **opsonin**, from the Greek, meaning to prepare the banquet. The opsonic power of the blood or more strictly speaking, the blood plasma, varies greatly from time to time. This power is expressed by a number which Wright named the **opsonic index**. It is the average number of bacteria engulfed by leucocytes under standard conditions of observation in the presence of blood serum preparations. A possible explanation of opsonic power is found in the clumping or agglutinating of the bacteria because of the chemical composition of the blood plasma. When the bacteria are thus massed together, a leucocyte coming in contact with them is enabled to engulf several at one time instead of taking them singly.

The leucocytes exert other phagocytic effects. Clark and Clark have shown that under normal conditions of development, leucocytes consume tissue materials, such as those of the tadpole's tail, which are absorbed and disappear during metamorphosis.

The rôle of the leucocytes during the absorption of fats from the intestine was indicated above. A considerable proportion of the absorbed fat is transferred from the absorbing cells into the lacteals by leucocytes which carry the fat, very finely emulsified, in their cytoplasm.

The production of plasma proteins is another function attributed to the leucocytes. Experimental observations show an abundance of leucocytes is required for a regeneration of fibrinogen in the blood after this protein has been depleted by removing a large proportion of the blood of an animal, defibrinating it and restoring it to circulation. So long

as leucocytes are present in abundance, fibrinogen is quickly regenerated. Moreover, high fibrinogen content of the blood has been repeatedly observed to accompany leucocytosis. Though not amounting to proof these observations indicate that fibrinogen is produced by leucocytes. The apparent rôle of the leucocytes in fibrinogen production suggests that they are also concerned in the formation of other plasma proteins.

The Life History of the Corpuscles.—Erythrocytes are produced by a process called *hemopoiesis*. This occurs chiefly in the bone marrow, in specialized cells of the hemopoietic tissues. During embryonic life, the liver is hemopoietic but its cells which have this function lose it at a later period of development. Probably the liver does not produce red corpuscles in any fully developed animal. The spleen also possesses hemopoietic power during early stages of development. This organ may even resume this function again after severe anaemia in the adult and steadily maintains hemopoiesis throughout the life of some species of animals.

The process of the formation of erythrocytes includes the formation of budded off daughter cells from large erythroblasts of the bone marrow. The newly formed buds appear to be nucleated at first but before they become actually free to enter the general circulation they lose their nucleus and acquire hemoglobin. The fate of the nucleus has not been discovered. It either degenerates or is extruded from the cell. In embryos and in adults that are rapidly producing red corpuscles as in the case of regeneration of blood after hemorrhage, some of the erythrocytes in the circulating blood are nucleated.

Non-nucleated erythrocytes do not have a long life. Some of them are disintegrating constantly. The daily production of bile pigment is an index of the uninterrupted destruction of hematin. This can come only from disintegrated red corpuscles. The amounts of bile secreted per day in human beings has been estimated as explained above. Computing from the average content of bile pigments in the bile, the amount of the daily destruction of hematin has been estimated. The number of red corpuscles which would yield this quantity of hematin can be computed from the figures for the average quantity of hemoglobin in corpuscles. In this way the daily destruction of corpuscles has been estimated. Conservative estimates show that about 5 per cent of all the red corpuscles of the body are destroyed each day. Higher estimates indicate that as much as 10 per cent of all the corpuscles are destroyed daily. If the latter estimate is a fair one, the average life of human erythrocytes is only ten days. The location at which this destruction occurs is largely in phagocytic cells of the liver. These are called Kuppfer cells. They exist in the endothelium along certain of the blood vessels of the liver and protrude strands of their protoplasm into the blood stream. These protrusions engulf erythrocytes as do pseudopodia of an amoeba. Some of the

erythrocytes are disintegrated, probably, in the spleen. That any erythrocytes normally go to pieces in the general blood stream, having their contents later on picked out by the liver, has never been satisfactorily demonstrated.

The numbers of red corpuscles in the blood of human beings varies with sex (average 4,500,000 per cu. mm. in women, 5,000,000 per cu. mm. in men), also with age, being more abundant in the infant than in older persons, also with conditions of health, with the diet and with habits of living. The number of red corpuscles varies especially with altitude or more strictly speaking with atmospheric pressure. Marked increases occur upon ascent of a mountain, the abundance being nearly doubled in some cases. This effect has been alleged by some writers to be due to a decrease in the amount of plasma in the circulation but this idea has been proved to be erroneous at least in cases where animals and men remain at high altitudes during several days or longer periods. A real increase in the total number of corpuscles then occurs. It is possible, however, that the sudden increases that occur during balloon ascensions and other exposures of only a few hours to low pressure are more apparent than real and are due to changes in the concentration of the blood.

The white corpuscles have not been so successfully studied as regards their life history. Probably the different kinds of white corpuscles are produced in different parts of the body. The lymph glands and the cells called myeloblasts of the bone marrow have been especially designated as sources of leucocytes. Probably both of these sources are active. The final fate of the leucocytes has only recently been investigated. They sometimes rapidly decrease in the blood and they are probably made way with constantly under normal conditions. Large numbers of lymphocytes are known to escape from the blood and get out upon the surface of mucous membranes especially those of the intestine. Lymphocytes are known to disintegrate under certain pathological conditions in the lymph nodes.

The Regulation of the Composition of the Blood.—The blood is constantly receiving material from numerous sources. Chief among these sources are absorption through the walls of the intestine, absorption of oxygen through the lungs and excretion of waste products from actively metabolizing cells, such as those of muscles and the liver. Minor sources include the glands of internal secretion, which contribute substances in small amounts but of great physiological importance. In the last analysis, cells of every type in the animal body contribute something to the blood. At the same time, the blood is constantly losing material. Through the lungs, gases are removed; through the kidneys, water, inorganic salts and numerous dissolved waste products are removed; through the liver and the intestines, some wastes are excreted; while every cell of the body is continually drawing upon the blood for its supply

of organic nutrients, inorganic salts, water and oxygen. Yet in spite of these numerous different processes, adding to and withdrawing from the blood, its composition remains remarkably constant under normal, healthy conditions. Fluctuations in the physical characteristics and chemical composition of the blood are confined within limits that are nearly as narrow as are those which mark the extreme variations in protoplasm itself.

The *reaction* of blood was discussed in a previous chapter. Variations in the pH of the blood are confined, usually, within the limits 7.33 to 7.38. Although this is due very largely to the buffer action of the bicarbonates, phosphates and proteins of the blood, it is also due in large measure to the fact that CO₂ is excreted from the lungs at a rate proportional to its concentration in the blood and to the fact that potentially acid or basic substances tend to be excreted by the kidney at a rate proportional to their concentration in the blood.

The *osmotic pressure* of the blood is also kept very nearly constant. This occurs in spite of various circumstances tending to disturb the osmotic pressure of the blood. The rate of water absorption from the intestine varies enormously. The rate of water excretion through the lungs and the skin is also variable and is not under regulation in accordance with the composition of the blood. Practically all of the work required for adjustment of osmotic pressure and for the maintenance of its constant value is thrown upon the kidneys. By variation in the relative amounts of water and solids, which they excrete, the osmotic pressure of the blood is kept within the narrow limits described in Chap. VI.

The *inorganic constituents* of the blood are likewise maintained by the kidney in a very constant concentration in the blood. This is especially true of sodium, potassium, calcium and magnesium. The significance of the maintenance of balanced proportions and constant concentration of these constituents was explained in Chapter VI.

The *sugar* content of the blood is maintained at a remarkably constant level. Any rapid intake of sugar from the intestines is followed by a compensatory storage of glycogen in the liver and to a less extent in other organs. Large amounts of sugar may be injected slowly into the blood without any corresponding increase in the sugar of the blood. The cells of the body take up sugar from the blood in proportion to the concentration of sugar and thereby maintain an equilibrium between the sugar in the blood and that in the cells. Any very great and sudden increase in sugar of the blood, an increase which may follow rapid injection of sugar or the eating of large amounts of candy, may so disturb this equilibrium as to cause a temporary increase in the concentration of blood sugar. Under these circumstances, the excess of sugar is rapidly excreted by the kidney so that the concentration is brought down to its normal value. If sugars other than glucose are absorbed from the intestine or are

injected into the circulation, they, too, quickly disappear from the circulation, because they are either absorbed by tissue cells or are excreted by the kidney. It thus comes about that the blood always contains about 0.1 per cent of glucose, normal variations being confined between 0.08 and 0.12 per cent, and other carbohydrates are absent from the blood except in such minute traces that their chemical identity has not been established. When the carbohydrate supply of the food is inadequate or during starvation, the blood sugar is still maintained at the normal concentration. This is effected by means of the breakdown of glycogen in storage cells. It is hydrolyzed to glucose and paid out from the cells into the blood as required. Proteins (that is, amino-acids) and glycerol serve, to some extent, as a further source of glucose in the body. The utilization of sugar is subject to very wide fluctuations as was clearly implied in the discussion of its preponderating rôle as a source of energy for muscular contraction. Very heavy muscular exertion may cause a temporary breakdown in the perfection of the regulation of blood sugar concentration, but this occurs only in cases of exhausting muscular work or extreme emotional excitement. Under all normal circumstances, however, the regulation of blood sugar is successfully maintained in spite of variations in activity ranging from complete rest in bed up to heavy muscular work.

The *fat* of the blood is likewise maintained at an extremely low but very constant concentration. The faster fat is poured into the blood from the thoracic duct, the faster it is removed by the fat-storing cells in the adipose tissue, by liver cells, by the leucocytes and possibly by other tissue cells. During the use of a fat-free diet or during starvation, the stored fat is gradually paid out into the blood so as to maintain a very small but constant proportion of fat or fat-like substances in the blood.

The *proteins* of the blood are maintained at remarkable constancy of concentration and of molecular individuality. Any foreign protein in the blood, if not sufficient to be fatal, quickly disappears, in some as yet unexplained manner. Moreover, the amino-acids of the blood are always present in a small but fairly constant proportion. Like glucose, they serve as a constantly available food for all the cells of the animal. Although not used for energy production, as generally as is glucose, they are required apparently for repair of protoplasmic constituents of all cells. Such evidence as is available, indicates that each one of the 18 or 20 different amino-acids is maintained at a practically constant concentration in the blood stream—and this in spite of great variations in the supply of amino-acids furnished by the digestion of the different proteins of a varied diet. Any tendency to increase of any one particular amino-acid results, apparently, in a corresponding increased rate of destruction of that one. This destruction occurs largely in the liver but it can probably occur in any body cells. An excessive amount of any one amino-

acid absorbed from the intestine or injected into the circulation quickly disappears showing that it is absorbed by the cells from the blood in proportion to its concentration. During starvation or inadequate protein feeding, the various amino-acids are still maintained in the blood for a time at least. The very noticeable degeneration of muscle tissue during advanced stages of starvation is an indication that the proteins of muscle cells are broken down to yield an amino-acid supply to the blood which permits it to nourish vital tissues such as the heart and the nervous system.

The Formation of Lymph.—Through the walls of the blood vessels, especially those of the capillaries, a constant exchange between the blood and tissue cells is maintained. The lymph is the medium across which this exchange occurs. It has been called the "middleman" of the body. Its constituents are derived partly from the blood and partly from the tissue cells but in the main, it is composed of materials of the blood plasma which pass through capillary walls. The nature of the process by which this passage is effected has been the subject of much controversy. One school of investigators, lead by Heidenhain, maintained that lymph was secreted by the cells of the capillary walls. Another group of workers, of whom Starling has been the most prominent, maintain that lymph is produced by a process of filtration and diffusion of plasma through the capillary walls. The latter theory has most in its favor. The blood is under pressure produced by the heart beat. This pressure might well be expected to force the fluid part of the blood, the plasma, through the microscopically thin walls of the capillaries. This seems especially probable in view of the fact that these walls are so constructed as to permit leucocytes to force their way through during diapedesis. The chief difficulty in the way of this idea that plasma passes into lymph spaces by a simple physical process nearly equivalent to filtration, is the fact that the amount of lymph produced is not always proportional to the changing amount of blood pressure. The amount of lymph produced can be estimated from the swelling of tissues and organs and from the amount flowing out of a fistula in the thoracic duct of an experimental animal. The lymph spaces of all tissues communicate with lymph vessels or channels which carry the lymph away from all the organs of the body. From all parts except the head, most of the lymph empties into the thoracic duct. That from the head region moves by way of a corresponding duct in the neck. As these ducts deliver lymph into the venous system, all the lymph is kept in slow but steady circulation. The chief force for maintenance of this slow movement is the pressure developed in lymph spaces by the processes of lymph production. It is therefore difficult to understand why lymph volume and rate of flow is not proportional to the pressure of the blood as it enters the various organs through small arteries. This difficulty has been met, however, by observations which clearly indicate that the permeability of capillary walls is

subject to great changes. Moreover, they are able to distend or constrict and thereby alter the pressure of blood within the capillaries without any direct dependence upon the pressure conditions in the arteries. Marked increases in the permeability of capillary walls can be produced in the frog's leg by heating it to 56°C. This results in great swelling by increased lymph production in the heated leg. Any such abnormal production of lymph is called **oedema**. Oedematous conditions can also be produced when a frog's leg is perfused with a salt solution. This occurs irrespective of whether the solution is of the same osmotic pressure as the fluids of the frog's body (isotonic) or is hypotonic. Moreover, the injection of foreign proteins, especially proteoses, into the circulation of any animal causes oedema. Extracts of crab meat and of the tissues of various other invertebrates also causes oedema. Substances producing this effect are called **lymphagogues**. In every case, they so increase the permeability of capillary walls that filtration and diffusion into lymph spaces becomes abnormally rapid. A peculiarly interesting and significant instance of this effect occurs after massive wounds which involve extensive bruises and lacerations. These result in the condition called *wound shock*. Its chief symptom is a great fall in blood pressure owing to a diminution of the volume of blood in the arteries and veins. Its immediate cause is the enlargement (relaxation) and therefore increased capacity of the capillaries. But this condition is accompanied by an increased permeability of the capillary walls so that physiological salt solution, if injected into the circulation, can only temporarily increase blood pressure to normal effectiveness. The salt solution quickly diffuses out of the blood system, permitting fall of blood pressure and return of other shock symptoms. The method used to overcome this condition by gum arabic solutions was described in Chap. VI. The fact that this colloidal material does not diffuse through the blood vessels and maintains osmotic pressure sufficient to prevent undue flow of water from the blood into the lymph spaces indicates that osmotic forces as well as mere filtration processes must be concerned in the regulation of lymph production.

The Composition of Lymph.—Lymph closely resembles blood plasma in general appearance and in composition. It contains all the plasma proteins though not necessarily in the same concentration. Fibrinogen is sometimes distinctly less abundant in lymph than it is in plasma. Lymph clots, but usually more slowly and less firmly than blood. This may be due to lack of platelets as the latter have not been found in lymph. White corpuscles are abundantly present in most specimens of lymph. Waste products, produced by tissue cells, are more concentrated in lymph than in plasma. This is what might be expected because the lymph is in contact with the cells. Because of these waste products, the lymph possesses an osmotic pressure slightly higher than that of the blood.

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CHAPTER XIX

THE CIRCULATION OF THE BLOOD

To serve as a common carrier, blood must be kept in constant motion. The flowing of blood is perfectly apparent when a blood vessel is cut or when a vein near the skin is so obstructed that it swells with accumulated blood. But the complete circulation of blood is not so obvious and was unknown to early students of physiology. During many centuries, the presence of blood in the arteries of the animal was doubted or positively denied. Blood was supposed to be only in the veins. Even when the arteries were demonstrated to be blood conduits, the blood was supposed to ebb and flow in both arteries and veins rather than to actually circulate. No channels for the flow of blood from arteries into veins could be demonstrated until the microscope was used to detect capillary vessels. All the more credit should be given to Harvey for his essentially correct explanation of the circulation (1628) in that he gave it before microscopes were available. The capillary circulation can be seen with the microscope very easily in any transparent, thin, animal structure. The web of the frog foot is frequently employed for this purpose. Small transparent animals such as embryos or very young fish, can be placed under the microscope in water containing an anaesthetic to prevent their movements and thus an opportunity is gained to observe the capillary circulation in various parts of their bodies. Mesenteries or other tissues of anaesthetized or pithed animals can be placed under the microscope in such a way as to enable the capillary circulation to be observed. Having knowledge of the capillaries, the modern physiologist can define the blood circulatory vessels as a system of closed tubes through which the blood is kept in motion by the pumping action of the heart.

The General Course of the Circulation.—The system of tubes includes (1) arteries which are the main vessels conveying blood away from the heart, (2) arterioles which are the small arteries distributing blood from the main channels into the different parts of the various organs of the body (3) arterial capillaries which are the small tubes branching from the arterioles, (4) capillaries proper which convey the blood in very thin streams through the tissues, (5) venous capillaries which convey the blood into (6) venules which are the small veins within the organs and (7) the veins which convey blood from the organs back to the chambers of the heart, except in the case of the hepatic portal vein which conveys to the liver and the renal portal vein, which occurs in amphibia and which conveys to the kidneys.

The comparative anatomy of the circulation shows that the above is essentially typical of the arrangement in all forms that are sufficiently complex in organization to possess a true blood circulation. The chief differences found in different circulatory systems are in the character of the pumping or propulsive mechanism. In some of the annelids, certain of the blood vessels possess the power of automatic, rhythmic contraction of their muscular walls. They are thus able to keep the blood in motion in the absence of a heart. Where this pulsating power is markedly confined to a definite portion of the circulatory system, this part might be said to be a rudimentary heart. An example of this is found in amphioxus. The simplest form of a heart is that of a tubular organ provided with valves at each end to direct the flow of blood from veins into arteries. This form is found in many of the crustacea and is exemplified by the heart of the crab. In animals which possess this rudimentary heart, the veins and arteries, immediately connected with it, are usually capable of pulsating so as to assist the heart in moving the blood. A slightly more complex heart is one with two distinct chambers, an auricle and a ventricle. The auricle receives the blood from the veins and the ventricle forces it out into the arteries. The fish heart is of this type. In such hearts the sinus venosus, a chamber into which the large veins empty, has some pulsating power and thus helps to force blood into the auricle. The bulbus arteriosus, the heavy walled and muscular arterial stem, is also rhythmically contractile and thus aids the ventricle in forcing blood into the arteries. Both the auricle and the ventricle are provided with valves so arranged as to permit positive force pump action. The amphibian heart is three chambered, having two auricles and one ventricle. One auricle receives blood from the pulmonary circuit, the other from the general circulation and is assisted by a sinus venosus. The single ventricle forces blood into the arteries of both the pulmonary and the general circulation and is assisted by a bulbous arteriosus. The reptilian heart is somewhat similar to the amphibian but possesses two chambers in the ventricle. These are not completely separated from each other, however, so that the oxygenated blood from the lungs is mixed, to some extent, with non-oxygenated blood from the general circulation as the blood leaves the ventricle. In birds and mammals the heart is four chambered. Sinus venosus and bulbus arteriosus are not present except in vestigial and comparatively non-functional form. The left auricle, receiving blood from the pulmonary veins, empties into the left ventricle which forces blood into the general circulation. The right auricle receives blood from veins of the general circulation and empties into the right ventricle which forces the blood into the pulmonary arteries. Birds and mammals thus have two complete and entirely distinct circulations, the general or systemic and the pulmonary.

The Rate of Blood Flow.—The blood flows fastest in the aorta and more slowly in the arterioles. This can be shown in animals by measurements of the volume of blood flowing from a severed artery per second. If this outflow is computed per unit of cross-section of the artery, the rate of flow can be estimated. Another method is based upon the use of

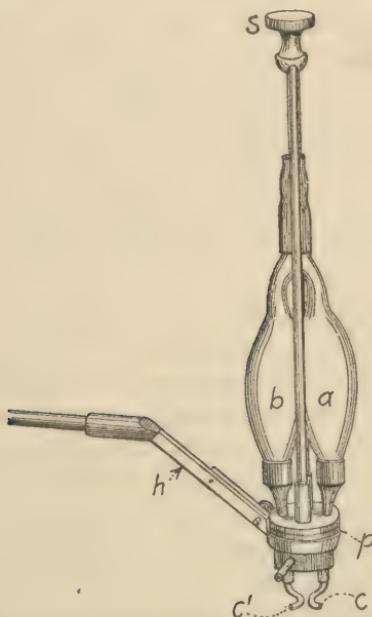


FIG. 142.—Ludwig's stromuhr. *a* and *b*, glass bulbs. *a* is filled with oil while *b* and the neck connecting the two bulbs are filled with isotonic salt solution. *p*, rotating plate by means of which the bulbs can be turned through 180 degrees. *c* and *c'*, canulas to be inserted into the free ends of a severed artery. *s*, thumb screw for rotating the bulbs. *h*, holder. When in place, the clamps on the artery are removed, blood flows through *c* into *a*, driving out the oil and forcing the salt solution into the artery through *c'*. When the blood in *a* reaches a graduation mark indicating 5 cc., the bulbs are turned through 180 degrees so that *b* lies over *c*. The blood flows into *b* and drives the oil back into *a*. When it just fills this bulb, they are again rotated and so on. Each filling of a bulb is equivalent to the outflow of 5 cc. of blood. When the instrument has been turned ten times, for example, 50 cc. of blood have flowed from the artery. Knowing the time and the calibre of the artery, the velocity of the blood flow can be calculated. (From Howell's "Text Book of Physiology," W. B. Saunders Co.).

the **stromuhr**, shown and described in Fig. 142. The flow of blood in the capillaries is comparatively slow, of the order of 1 cm. per second. This fact is established by observations on the rate at which corpuscles are seen to move through capillaries of living tissues under the microscope. The blood flow is faster in the venules than in the capillaries and in the large veins it flows almost as fast as in the large arteries. The explanation of these differences in rate of flow is found in the capacity of the several kinds of blood vessels. Capacity in this sense is comparable to the width of bed of a stream. The wider the stream bed, other things being equal, the

more slowly water flows through it. The current in a pond is much slower than that in its inlet or its outlet. The pond has a much greater capacity on account of its wide stream bed. It is difficult, at first thought, to realize that the capillary blood stream bed is wider than that of the arteries. It turns out that such is the case, however, because when any blood vessel branches, the cross-sectional area of the two branches is greater when added together than that of the vessel of which they are

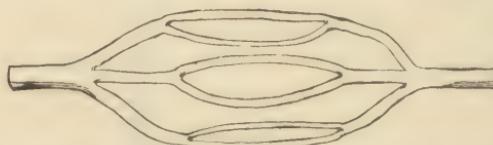


FIG. 143.—Diagram to indicate that the capillary blood stream is wider than the arterial or venous.

branches. This general idea is indicated in Fig. 143. The only reason why blood does not flow quite as fast in the vena cava as it does in the aorta is that the cross-section of the vena cava is slightly larger than that of the aorta. The rate of flow in veins is steady, that in arteries fluctuates with each heart beat, increasing somewhat during each contraction of the ventricle. The effect of the heart pulsations can be seen even in the arterial capillaries, for in them the blood corpuscles are observed to move faster and then slower in response to each heart beat. These pulsatory changes in rate of flow are lost after the blood has passed through the narrow capillaries into the venous side of the circulation.

The Time Required for a Complete Circulation.—The time consumed in the complete circulation of a drop of blood can be estimated from the capacity of the ventricles, that is, the amount of blood which they put out at each contraction and the total amount of blood in the body. A more direct method and one applicable to human beings involves the injection of some innocuous foreign substance into a vein on one side of the body and measurement of the time elapsing between the injection and the moment when the foreign substance can be detected in the blood of the corresponding vein on the opposite side of the body. To reach this latter point the substance must have gone to the heart, passed out of the right ventricle through the pulmonary circulation and back to the heart, gone out of the left ventricle, through the arterial circulation and the systemic capillaries and so reached the veins. Potassium ferrocyanide, which can be detected in very small amounts, has been utilized for this experiment. Other methods have been employed, but they depend upon the same general principle.

The actual results observed will be determined, to some extent, by the length of the blood circuit measured, that is, by the location of the veins that are injected and observed. The longest circuits are those

through the extremities of the body; the shortest is the coronary which passes through the heart tissues. Excluding the coronary circulation, the average time for the complete circulation of the blood through all the other circuits of the adult human body is about 23 seconds. This figure serves to give a general idea of the rapidity with which substances getting into the blood are distributed over the body. Another striking statement of the same idea is often made by saying that all the blood in the human body is completely circulated twice per minute. The time of the complete circulation varies somewhat in animals of different species and in the same individual under different circumstances in accordance with the rate of the heart beat, the contraction and relaxation of blood vessels and some other factors.

Blood Pressure.—Liquids flow from a place of higher pressure to one of lower pressure. In any passive tube, that is, one which is not contracting or expanding, a liquid flowing through it is under pressure which steadily diminishes as the liquid progresses. This is demonstrable by measurements of the heights to which the liquid will rise in vertical tubes opening off from the main one at different points. These heights show a progressive diminution. The very fact of the circulation of the blood shows that it is under pressure which is constantly highest in the arteries and lowest in the veins. Otherwise the blood would not continue to circulate. Blood pressure may be defined as the pressure which the blood exerts against the walls of its containing vessels. It is theoretically measurable in any given blood vessel by the height of a column of blood which the pressure in that vessel would hold up in a tube connected to it. The gentle outflow of blood from capillaries or veins ruptured by surface or shallow wounds shows that the pressure there is low. The violent upspurt of blood from a cut artery indicates a much greater pressure.

Arterial pressure was first measured by Dr. Stephen Hales, an English clergyman, who connected a long glass tube by means of a brass canula to one of the leg arteries of a horse. He observed that the blood rose in the tube to a height of nearly 9 feet and remained at approximately that level with fluctuations due to each heart beat. His work was reported in his "Haemostatics" in 1733. It marked an important advance because it was one of the earliest applications of the experimental method to physiological studies.

Methods of Measuring Blood Pressure.—The flow of blood into a vertical tube withdraws enough blood from the circulation to diminish the pressure appreciably. For this reason, the simple method described above gives unsatisfactory measurements. Another serious objection to it arises from the fact that the outflowing blood soon clots and so precludes continuous observations on the changes of blood pressure. For animal experimentation, the mercury manometer, devised by Ludwig and improved by other investigators, is satisfactorily used. The manometer

itself is a U tube containing mercury. One end of the tube is bent over and connected by thick walled rubber tubing to a canula suitable for tying into a blood vessel (Fig. 144). From the other end of the U tube, projects a vertical rod of which the lower end is connected to a float that rests upon the surface of the mercury.

The upper end of the vertical rod carries a cross arm which is provided with a writing point to bear upon a kymograph. The use of mercury instead of some lighter liquid greatly reduces the actual amount of motion of the manometer in response to a given change in pressure. This is a distinct advantage in most cases. The space between the mercury of the manometer and the blood at the mouth of the canula is filled, prior to the experiment, with a solution that checks blood clotting.

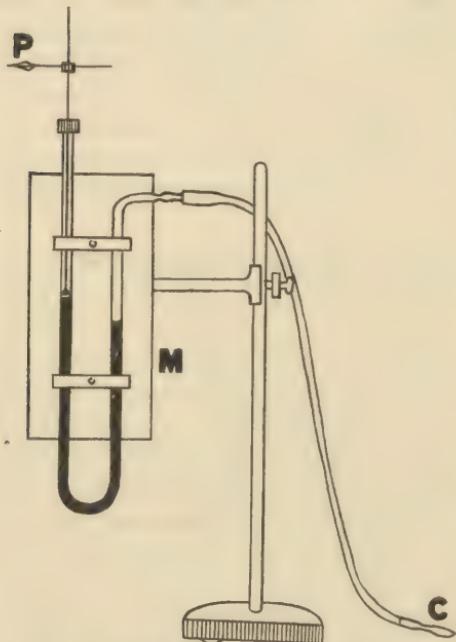


FIG. 144.—Blood pressure manometer. *M*, mercury manometer in form of a U tube. *P*, writing point for tracing the record. *C*, canula to be fastened in the artery. Pressure tubing connects the canula to the manometer.

pulse in the artery beyond the compressed region or is gradually decreased until the previously obliterated pulse becomes just barely perceptible. The pressure can be applied most conveniently to the upper arm while observations are taken by feeling the pulse in the ordinary way in the radial artery at the wrist or by means of an automatic recording device, a sphygmograph (p. 541), attached to the wrist. The application of pressure to the arm is effected by pumping air into a rubber bag contained in an armulet strapped on above the elbow. The bag is connected to a mercury manometer which measures the air pressure. The pressure just sufficient to entirely obliterate the pulse measures the maximum pressure in the artery, the so-called systolic pressure.

Cause of the Sustaining of Blood Pressure.—The force of the heart contraction, or more strictly speaking, the contraction of the ventricles, is the cause of blood pressure. But this force alone would not furnish a

For observations on human beings, a different principle must be employed. This principle is the application of pressure to the skin in the region of an artery. Pressure is either gradually increased until it is just sufficient to obliterate the

sustained pressure if unaided by other factors. A liquid pumped into passive, that is, rigid tubes of uniform bore is under alternatingly high and low pressures corresponding to each stroke of the pump. The blood actually flows steadily through the capillaries, indicating that it is under a sustained pressure. The conversion of the intermittent flow from the heart into a constant flow in the capillaries is due to several factors. Two are of paramount importance. One of them is the pressure which arterial walls exert upon their contents. This is partly due to the elasticity of the arteries. They are comparable, in a sense, to elastic rubber tubes which, under distension, press by elastic recoil upon fluid contents. In addition to the recoil of stretched elastic tissues, the walls of arteries possess a further mechanism in the form of muscular tonus. The non-striated muscles which constitute a prominent part of the wall of every artery maintain some degree of tonus throughout life. This sustained contraction, though varying from time to time in accordance with excitation of these muscles, is at all times a considerable factor in the sustaining and regulating of arterial blood pressure. A second important factor in the sustaining of arterial pressure is the resistance to the passage of blood through the small blood vessels, especially the capillaries. This factor is commonly called the **peripheral resistance**. The cause of this resistance is, in the main, friction produced by the passage of the somewhat viscous blood through minute tubes. The fact that such resistance brings up liquid pressure in a tube is familiar to every one who has observed how cutting down of the nozzle opening at the end of a garden hose increases the pressure under which the resulting smaller stream of water emerges. Peripheral resistance is a requisite factor for the maintenance of an effective blood pressure. This fact is made apparent by the disastrously low arterial pressures which result in certain pathological conditions from a wide-spread dilatation of arterioles and capillaries.

Other minor factors modify blood pressure. One of these is breathing. Each time the thorax enlarges (inspiration) some suction is exerted upon all the blood vessels within the thorax, especially upon the large veins near the heart. The veins are affected more than the arteries because veins possess comparatively less sturdy and elastic walls. Conversely, each time the thorax decreases in size (expiration) a tendency to increase of pressure upon thoracic blood vessels results. The flow of blood and the pressure in the pulmonary circuit are considerably modified by respiration. Pressure in the arteries of the entire body rises and falls slightly with each breathing movement although neither rising nor falling phase of pressure is exactly synchronous with either inspiration or expiration. These pressure changes are nevertheless timed according to the breathing. Another modifying factor is gravity. This force tends to increase pressure in arteries at levels below that of the heart and to decrease pressure in arteries at levels above that of the heart. This fact explains

the efficiency of lying down to prevent faintness caused by pressures that are insufficient in cerebral arteries to maintain an adequate blood supply for the brain. A third of these accessory factors is the actual volume of blood in the circulation. Other things being equal, pressure tends to be proportional to the quantity of blood in the system.

The Curve of Arterial Pressure.—The curve as shown in Fig. 68 indicates certain small waves due to individual contractions. These are superimposed upon larger curves, the respiratory waves of blood pressure. Still longer curves are often seen. Sometimes they are rhythmic in character, but more often are merely temporary changes in the general level of pressure in the artery under observation. They are due, in general, to vasoconstriction and relaxation. This is especially true when rhythmic waves of blood-pressure changes occur. These are the so-called Traube-Hering waves. They result from rhythmic changes in the tonic activity of the vasoconstrictor center in the medulla. In some cases, changes in blood pressure are due to altered peripheral resistance, that is, contractions or dilatations of arterioles and capillaries. In still other cases, changes in the general level of blood pressures are the results of changes in the rate and force of the heart beat.

Systolic and Diastolic Pressure.—At the height of ventricular contraction (ventricular systole), pressure in the arteries reaches a maximum. This is the systolic pressure. During the relaxation of the ventricle (ventricular diastole), blood pressure tends to fall. It reaches a certain minimum just before the beginning of the next succeeding systole. This minimum is called the diastolic pressure. The difference between these two pressures gives an index of the capacity of the circulatory system to sustain pressure. An abnormally great difference between systolic and diastolic pressures may result from: (1) A decreased volume of blood, (2) a lowered peripheral resistance, (3) a decreased tonus of arterial muscles or (4) a decreased elasticity of arterial walls. The last of these causes has considerable interest in human physiology. This is due to the all too frequent occurrence of hardening of the arteries, arterio-sclerosis. The calcification of arterial walls, producing this condition, tends to occur with advancing age and becomes a serious and dangerous condition in a surprising proportion of people past middle age. The difficulty comes about through the comparative rigidity of calcified arteries. The resulting great fluctuations in arterial pressure cause a strain upon the heart and indeed upon the entire circulatory system.

The Amount of Blood Pressure.—In the adult human being the actual pressures observed for the large arteries of the arm normally vary between 135 and 150 mm. of mercury. The pressure is less in the smaller arteries and is larger in the aorta. The pressure varies with sex, averaging higher in men than in women. It varies during muscular or mental work. It shows a tendency to fall in fatigue, especially if it be extreme.

Numerous other conditions, both physical and psychical, cause more or less fluctuation of a temporary character.

The general level of blood pressure varies with the species. There is a general tendency for blood pressure to vary with the size of the animal, although this relationship has not been found to be an exact one. The following figures compiled by Volkmann and Nikolai show pressures measured in the larger arteries of some mammals.

	MM. HG.		MM. HG.
Horse.....	180	Goat.....	130
Calf.....	160	Cat.....	110
Sheep.....	160	Rabbit.....	100
Dog.....	140	Guinea-pig.....	85

The Capillary Pressure.—Pressure in the human capillaries is roughly measured by finding the weight which, suspended from a piece of glass resting upon the skin, is sufficient to make the skin blanch. The weight per sq. mm. of skin upon which the glass rests is taken as an index of the capillary pressure. It is so taken because the weight is just sufficient to obliterate the lumen of the capillaries and thus overcome the pressure in them. Similar methods are used to measure pressures in the capillaries of transparent animal membranes. Pressures found by this method are only approximate. When the weight per sq. mm. is translated into the heights of corresponding columns of mercury, the figures obtained vary between 18 and 40 mm. of mercury in mammals. In short, the pressure in the capillaries is much lower than in the arteries.

The Venous Pressure.—Pressure in the veins can be measured in animal experimentation by methods analogous to those used for arteries. The manometer used, however, is commonly filled with water or a water solution instead of mercury because this procedure gives accuracy in the measurement of the small pressures that are found in veins. In human beings the pressure in veins near the skin is easily measured by finding the amount of compression required to stop the flow of blood through such a vein. The stoppage of flow can be detected by the collapse of the vein on the central side of the compressed point. The pressures observed in the veins of the limbs of mammals is usually less than 10 mm. of mercury and shows a fairly regular tendency to decrease along the venous system toward the heart. In the jugular vein, for example, pressures less than 1 mm. of mercury have been recorded. In the largest veins, inferior and superior vena cava, pressures are actually negative, that is, there is a suction instead of a pressure exerted upon the walls of the veins. This negative pressure is considerable (2 to 3 mm. of mercury) in the parts of the cavae nearest to the heart, parts within the thorax. The explanation of this negative pressure has been variously given. It is probably due to several causes. Of these the most effective is the suction action of the thoracic walls as they expand during inspiration.

This serves to draw blood from the capillary areas into the large veins and has its effects, as explained above, upon blood pressure in the arteries. Another cause of suction in the veins is the expansion of the auricles during their diastole. As they expand, they tend to suck blood out of the veins into the heart.

The Heart.—A certain continuity of heart structures has become the basis of modern explanations of the behavior of the heart. This continuity is very easily seen in the simple tubular or two-chambered heart of lower animals. The muscular structures in such hearts form a continuous layer in the walls of the heart and this layer is also continuous with

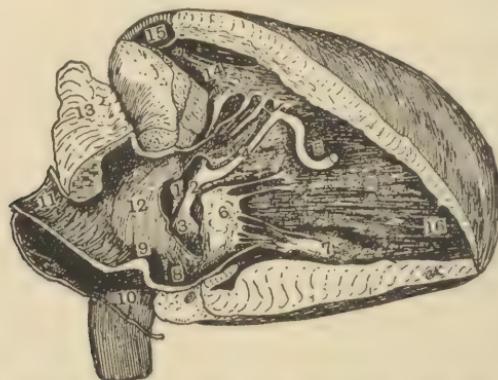


FIG. 145.—The bundle of His, as shown by dissection of the heart of the calf. 2, the bundle which divides into two branches, one entering the right, the other, the left ventricle. 3, the beginning of the bundle in the auricular septum known as the A-V node. 4, branch of the bundle entering the right ventricle. 1, central cartilage. (After Kieth, from Howell's "Text Book of Physiology," W. B. Saunders Co.).

the muscle layer of the sinus venosus and of the conus arteriosus which forms the root of the arterial system. For the hearts of many of the lower animals an equally unbroken, continuous system of nervous structures has been described. These structures are composed of a network of nerve fibers and minute ganglia. Such a continuity of the especially excitable tissues, muscle and nerve, insures a perfect conduction of excitation through the entire structure of the heart. This, as explained above, accounts for the ease with which the "all or nothing" principle can be demonstrated with heart muscle. Continuity of structure, however, is not easily demonstrated in the vertebrate heart. The connective tissues abundantly present at the auriculo-ventriculo junction or groove appear, to casual observation, to form a complete interruption of the continuity of the muscular and nervous structures of the vertebrate heart. But with more accurate observation, a connecting bridge of tissue between the muscle layers of the auricle and those of the ventricle can be seen. This bridge is composed of fibrous material which is sometimes called muscular

though, as a matter of fact, it is so modified that there is some question as to whether these fibers should be called muscular. The structure constituting this bridge, together with numerous other fibers which connect the bridge to different parts of muscles of the ventricle, is shown in Fig. 145. This structure is called the auriculo-ventricular bundle or the bundle of His. It undoubtedly conducts excitation from the auricle to the ventricle. Its proper functioning is requisite for a normal and orderly sequence of events in the heart beat.

The Cardiac Cycle.—By this term is meant the complete sequence of events during one complete heart beat. In describing this sequence, it is convenient to begin with the stage during which all the muscles of the heart are in a relaxed condition. This is called the period of diastole or rest of the entire heart. Although this complete diastole does not occur in the hearts of some species in which contraction begins in the part of the heart connected to the veins before the part at the base of the aorta has completely relaxed, yet in the mammalian heart and in some other types there is a period when all parts of the heart are simultaneously in diastole. Contraction or systole of the heart begins with the contraction in the sinus venosus in the lower animals but in a part of the right auricle at the sino-auricular junction, or node, in mammals. The contraction then spreads over the heart.

The manner of its spread can be observed with the unaided eye in the case of a slow-beating heart like that of the turtle or the frog heart cooled to about 10°C. It can be seen either in the exposed heart *in situ* or in the completely excised heart. Contraction of the sinus is followed by contraction of the auricles, then of the ventricles and finally of the base of the aorta. This sequence constitutes a wave of contraction spreading over the heart muscle. Observations with the unaided eye are confirmed and made more accurate by means of successive photographs of the exposed heart and by graphic records of the heart beat. To obtain these records, a series of levers attached by threads to the walls of auricles and ventricles may be used. But satisfactory results showing the wave of the heart contraction can be obtained by the use of a single lever attached to the tip of the ventricle. In this case, the so-called suspension method is used (Fig. 146). A tracing obtained by this method is shown in Fig. 147.

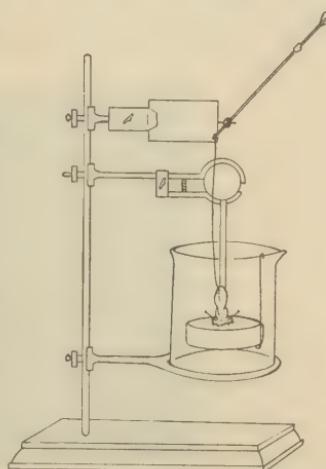


FIG. 146.—The suspension method for recording the heart beat. An excised frog heart is pinned to a cork suspended in a beaker of isotonic salt solution. A wire connects the tip of the ventricle with one end of a light, counterbalanced, straw lever of which the point writes the record.

Another method for the study of the cardiac cycle depends upon the measurement of pressures within the heart. Hollow tubes or sounds are thrust into the chambers of auricle and ventricle through the walls of the large blood vessels and are connected by flexible tubes to delicate manometers. These record graphically the momentary changes of intracardiac pressures. This method has been successfully employed in the study of the mammalian cardiac cycle (Fig. 148).

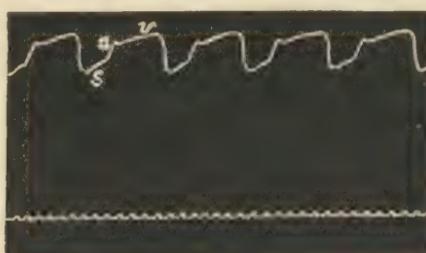


FIG. 147.—Record of the frog heart beat. *S*, contraction of sinus. *a*, contraction of auricle. *v*, contraction of ventricle. The time line shows 0.2 sec. intervals.

From the study of these records, it is shown that the auricles become filled with blood during their diastole. During the first part of auricular diastole the ventricles are in systole and the auriculo-ventricular valves

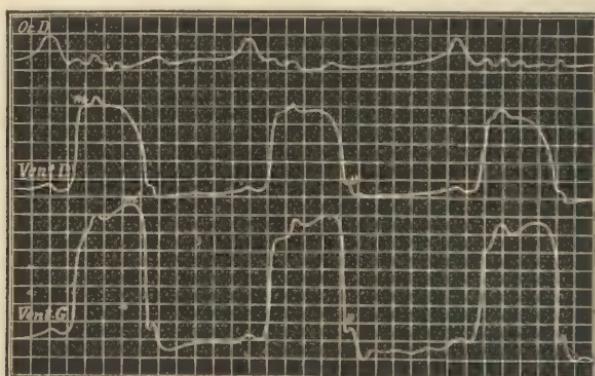


FIG. 148.—Record of the pressure changes in the heart of the horse. *Or D*, in the right auricle. *Vent D*, in the right ventricle. *Vent G*, in the left ventricle. These curves show, graphically, the detailed events of the cardiac cycle. (After Marey, from Howell's "Text Books of Physiology," W. B. Saunders Co.).

are closed. But as soon as the ventricles begin their diastole the auriculo-ventricular valves open and the blood flows into the ventricles. This passive filling of the heart is merely due to the momentum of blood flow in the veins and continues during the period in which the entire heart is in diastole. This period lasts during about half of the cardiac cycle of the human heart. For instance, a heart beating at the rate of 75 per

minute, so that each cycle lasts 0.8 sec., would be in complete diastole about 0.41 sec. This period is a smaller part of the cycle of the hearts of some animals. The end of the complete diastole or rest period of the heart is marked by auricular systole. This forces blood into the ventricle under slight pressure and gives the ventricle the greatest distension that it has during the cycle. At this time the flaps of the auriculo-ventricular valves float into their position of closure. The semilunar valves are at this time closed. The ventricular systole, following quickly after the auricular, brings up pressure in the ventricles above that in the auricles. Simultaneously, the auriculo-ventricular valves are tightly closed. Pressure on the blood in the ventricles continues to rise rapidly with ventricular systole. As soon as the intraventricular pressure exceeds that in the arteries, the semilunar valves open. The pressure continues to rise up to the point of maximum contraction of the ventricles. This pressure is transmitted into the arteries. During this period, the ventricle empties itself into the arteries. As soon as ventricular diastole sets in, the semilunar valves close. Very shortly after this, the release of pressure in the ventricles permits the opening of the auriculo-ventricular valves. Auricular systole has a duration less than half that of the ventricular systole. In the mammalian heart auricular systole is about one third the duration of the ventricular. The right auricle begins its systole before the left because the heart beat originates at the venous opening into the right auricle and then spreads over the heart. In the two ventricles, systole begins simultaneously.

The time of the cardiac cycle can be inferred from the rate of the heart beat. The number of beats or complete cycles per minute varies among mammals from about 25 in the elephant to about 175 in the mouse. In cold-blooded animals both vertebrates and invertebrates, comparatively slow heart rates are the rule. The average heart rate for human beings is usually given as 72 per minute. The heart rate of all animals changes very noticeably with changing conditions such as age, size, sex, temperature and reflex excitations. Emotional states have important effects upon the heart rate in mammals. Heart rates as high as 600 per minute have been observed in a frightened mouse.

The Heart Sounds.—During each cardiac cycle certain sounds can be heard by the aid of a stethoscope. Owing to the diagnostic value of the detection of abnormalities in the sounds of the human heart, their physiological significance has been extensively investigated. Two chief sounds can be heard during each cardiac cycle. The first of these is known to occur during ventricular systole. It begins simultaneously with the beginning of contraction of ventricular muscles and continues until they begin to relax. All muscles give off a vibratory sound during the contraction of their fibers. The powerful ventricular systole is the cause of this sound. Auricular muscles are too weak to give off any

perceptible sound during their systole. The second sound of the heart is known to occur simultaneously with the closure of the semilunar valves. The blood in the arteries, being under high pressure at this time, closes these valves with a sharp snap when ventricular pressure suddenly diminishes. The source of this second sound is shown by several observations. Experimental destruction of the semilunar valves in animals results in a failure of the second sound. Insufficiency of the semilunar valves sometimes occurs in human beings as a result of their partial rupture from overstrain or of the formation of rough spots after infections. Such

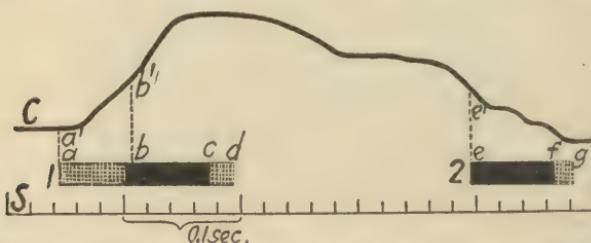


FIG. 149.—Graphic representation of the relation of the heart sounds to the ventricular systole. *S*, time record. *C*, record of contraction of the ventricle. 1, duration of the first heart sound. 2, of the second sound; the small letters mark the times at which differing intensities of the heart sounds are distinguished. (Einthoven and Geluk).

insufficiencies cause the second sound of the heart to lose its usual brief, sharp character and to become a murmur or gurgle. To what extent the closure of the auriculo-ventricular valves contributes to the first heart sound is uncertain. After their experimental destruction in animals the first sound of the heart is not obviously modified. These valves probably do not close suddenly enough to produce a perceptible sound. A third very faint heart sound has also been described. Its origin is not satisfactorily explained. Einthoven suggested that it is produced by after vibration of the closed semilunar valves. Other investigators have attempted to explain it as due to the opening of the auriculo-ventricular valves and the attendant sounds of blood running in from auricles to ventricles. No satisfactory diagnostic significance has been attached to the third sound. The relationship of the heart sounds to pressure changes in the ventricles is shown in Fig. 149.

The Electrocardiogram.—A graphic record of the electrical variations produced by the beating heart is called an electrocardiogram. These variations are the result of the development of electrical negativity of excited muscle as compared with unexcited tissues. The electrical variations of the heart are thus entirely comparable to the negative variation or action current of other muscles. When the skeletal muscles are at rest, save for quiet breathing movements, the action currents of the heart can be satisfactorily recorded and accurately measured by means of a string galvanometer. Such an instrument, when especially adapted for

observations on the heart, is called an electrocardiograph (Fig. 150). The movements of the string of the galvanometer are photographed upon a moving sensitive film to give the electrocardiogram. The changes of electrical potential in the heart can be communicated to the galvanometer

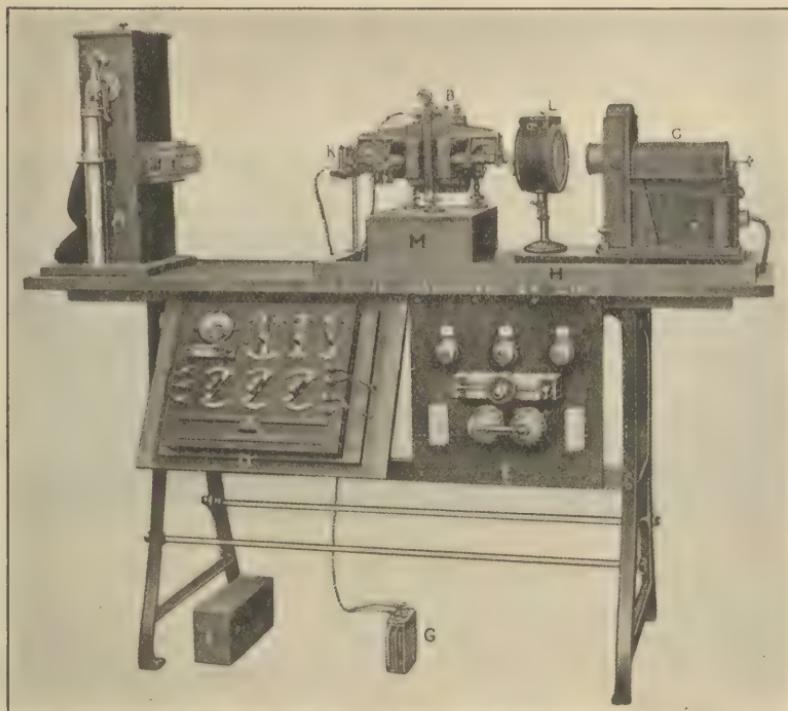


FIG. 150A.

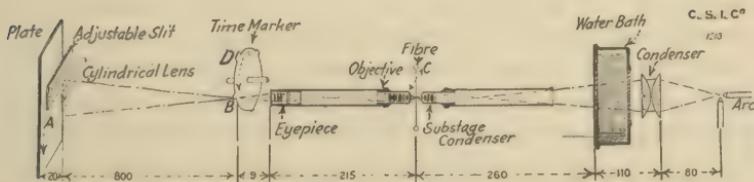


FIG. 150B.

FIG. 150.—The electrocardiograph. *A*, view of the instrument with accessories assembled. *B*, diagrammatic representation of the essential parts. (From the catalogue of the Taylor Instrument Co. Apparatus made by the Cambridge Scientific Instrument Co.).

through electrodes applied to the surface of the body. This is the case because animal tissues and fluids are able to conduct electric currents. Large non-polarizable electrodes are applied to the two hands or to one hand and one foot in order to connect the human body to the apparatus. In studies upon experimental animals small non-polarizable electrodes can be applied to definite locations of the exposed or excised heart. The

electrocardiograms that have been obtained by this latter method have been especially useful in studies designed to show the point at which the heart beat originates and to show the course which the wave of contraction takes as it progresses over the heart muscles.

An electrocardiogram varies somewhat in accordance with the species of animal, the condition of the heart and the place of application of electrodes to the heart or to the skin. A human electrocardiogram, taken with electrodes applied to the two hands (Fig. 151) will serve as

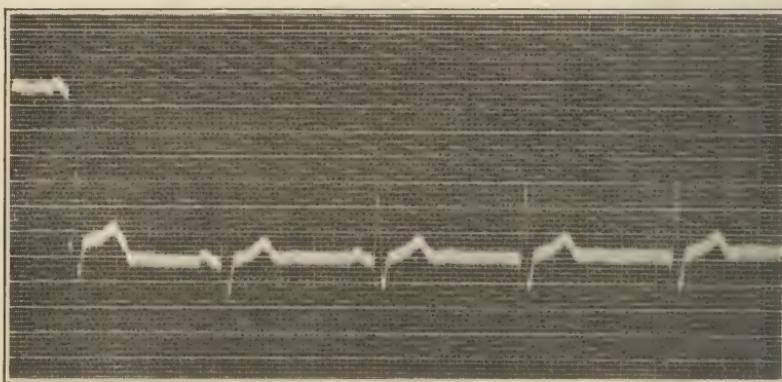


FIG. 151.—Electrocardiogram. Vertical lines show 0.05 sec. intervals, finest horizontal lines indicate electrical variations of 0.01 millivolt. At the left of the record is a deflection of the string caused by a measured voltage from a standardized cell. The record reads from left to right. The P, Q, R, S, and T waves are recorded for each of five heart beats. The R wave is the rapid and therefore faint upward curve showing an electrical variation of 0.12 millevolt. The Q wave, downward curve immediately preceding R, is very small.

the basis for a discussion of some of the main facts and theories derived from such records. During each cardiac cycle, the string of the galvanometer moves so as to produce five distinct waves or variations in the record. These are commonly known as the P, Q, R, S, and T waves. The P, R, and T waves indicate that the right side of the body (right hand) is electrically negative to the left side of the body (left hand), provided that the connections to the galvanometer are made in the usual standard manner. The Q and S waves indicate the reverse condition, negativity of the left side as compared with the right side. The P wave occurs simultaneously with systole of the auricles and registers the current of action of auricular muscles. A short interval between the P wave and the Q wave represents the time required for transmission of excitation from the auricles to the ventricles. The Q wave shows that some structure near the apex of the ventricle is electrically negative to the main parts of the heart. This observation is in agreement with the known distribution of the fibers which branch downward from the bundle of His. These fibers extend well down into the tip of the ventricle. The

exact structures which produce the Q wave have not been identified with certainty. Contraction of the papillary muscles probably account in part at least for this wave. These muscles pull down upon the fibers which lend support to the auriculo-ventricular valves. The R wave marks the spread of excitation over the main mass of ventricular muscle. As it shows negativity of the right side of the body with respect to the left, it indicates that the heavy musculature at the base of the ventricle is excited, in part at least, during the early stages of ventricular systole. The S and T waves have not been given a very exact interpretation. Both of them occur during the period of ventricular systole and indicate that with the changing shape of the ventricle during its contraction electrical negativity fluctuates somewhat. Both of these waves are subject to considerable variation in accordance with the condition of the heart and the manner of making the record. The S wave coincides approximately with the moment at which the tip of the ventricle is in its maximum state of contraction. The time relations of the different events which make up the sequence of the cardiac cycle can be accurately measured by the aid of electrocardiograms. It is for this reason that such records have been valuable in the study of the physiology of the heart and the diagnosis of its disturbances.

Automaticity of the Heart.—By this term is meant the power of the heart to initiate beats even when completely isolated from the rest of the body. Automatic rhythmic contractions occur even in minute portions of the heart muscle after they are cut off from the rest of the heart. This is similar to the independent excitability which is a property of all living matter; so that, in a sense, it is not peculiar to the heart. But, in another sense, it is peculiar in that the heart muscle appears to act rhythmically in the absence of any external stimulus. This is only an apparent anomaly, however, because some reaction between the heart cells and their surrounding nutrient medium must constitute the stimulus. This reaction takes the place of the external stimuli which commonly arouse the activity of other excitable tissues. Even the rhythmic character of heart muscular contractions is not characteristic of this organ; for other structures, such as striated and non-striated muscle and cilia, are also capable of rhythmic contractility. The beating of intestinal muscle was described above. This property, however, is so developed in the heart that it has attracted much investigation. Rhythmic automatic excitation must occur in the case of normal heart beats in some one limited region of the heart. This is apparent because the orderly sequence of contractions during each cardiac cycle shows that beats originate in the region of the heart where the veins join the auricle and that the contraction wave, spreading over the heart, is a continuous one which is due to the conduction of excitation rather than to independent excitations. Accordingly, investigation of the heart automatic rhythm is

narrowed down to three fundamental problems: (1) Where is the tissue in which the individual heart beats originate? This means what part of the heart is the "pace setter" for the rest of the organ. (2) What type of structure initiates the beat? Historically, this question has received two sorts of answers: that it is a nervous structure (the *neurogenic theory* of the heart beat) and that it is a muscular structure (the *myogenic theory* of the heart beat). (3) What is the nature of the so-called "internal stimulus," that is, what is the excitatory process?

Of the last of these questions, discussions have been presented above. These brought out the relation of the balance of the concentration of Na, K and Ca ions in blood to the beat (p. 149), the relation of C_H (p. 157), certain nutritive requirements (p. 150) and the significance of dynamic equilibrium in reversible reactions (p. 187). A summary of these discussions, indeed, of all studies of heart excitation can be expressed in the rather vague generalization that normal heart excitation is in some way dependent upon optimal concentrations of certain ions and of certain nutrients. A more definite theory must await the future development of the theory of excitation of living matter in general.

The "Pace Setter" of the Vertebrate Heart.—Stannius described an experiment in which a ligature was tied around the heart of a frog in such a way as to cover the narrow zone which marks the opening of the sinus venosus into the auricle. This zone is easily seen as a white line. It is called the *sino-auricular node*. Immediately after tightening of the ligature, pulsations in the auricle and ventricle cease. The sinus venosus continues to beat at the same rate as before. This observation indicates that the sinus is the originator of the heart beat and that the ligature interrupts the conduction of excitation from the sinus to the rest of the heart. It does not indicate, however, what particular part of the sinus serves as origin of the beat. Experiments, designed to delimit this region more definitely, have shown that some part of the sino-auricular node is the actual origin in most of the vertebrates that have been investigated. This is true for turtles, frogs, certain fishes and mammals. For birds, it is probably true but certain complications in the structure of the bird heart have made conclusions unsatisfactory. Numerous different investigators, among whom Lewis, Eyster, Meek and Erlanger have been prominent, contributed to these investigations. Several different types of experiments have furnished evidence.

In one kind of experiment, local cooling or heating of certain definite points of the heart is employed. This method can be used because the rate of the heart beat changes sensitively with changes in temperature. Temperature changes in one small localized region of the heart will not affect the rhythm of the entire organ unless this region is the "pace setter." It has been shown, for example, that heating or cooling when localized upon the surface of the auricle or ventricle has no effect upon

the heart rate. Such changes at the sino-auricular node do markedly affect the heart rate.

Another type of experiment includes the excision of portions of the sino-auricular node so that its "pace setting" cells will be removed from the heart. Similar experiments include cauterizing or clamping the node or treating it with formalin. These procedures destroy the functional activity of the nodal tissue. Results of all of them indicate that the sino-auricular node is the normal "pace setter" for the vertebrate hearts that have been so investigated.

A third type of experiment depends upon the timing of the electrical variations in different parts of the heart structures. The object of this procedure is to find the exact location at which the negative variation of a given cardiac cycle occurs first, in other words, to find what part of the heart is the first to become electrically negative to surrounding regions. This negativity is an index of excitation. The point which first shows it must be the originator of the cardiac cycle. This method yields the most detailed and probably most reliable results. Although these experiments show that different parts of the sino-auricular node serve as "pace setter" in different animals, they indicate the nodal tissue as the general locus from which the heart beat starts in all the vertebrates.

The Auriculo-ventricular Node.—The fibrous tissue, which constitutes the bundle of His and connects the auricle with the ventricle, was described above as the connecting path over which excitation is conducted. Under normal circumstances it probably functions only as a conductor. Much evidence has accumulated, however, to show that under some circumstances it serves as the point of origin of heart beats. When the sino-auricular node is cooled to such a temperature that its beating is below a certain critical rate, the contractions of auricles and ventricles take on an abnormal character. This abnormality is best recognized by measuring the time interval between the auricular and ventricular systoles. This interval is accurately measured on the electrocardiogram and is found to be abnormally short. This phenomenon is best explained by assuming that the beats originate in the auriculo-ventricular node and so are distributed to the ventricles almost as rapidly as to the auricle. This assumption is further substantiated by warming or cooling of the auriculo-ventricular node, a procedure which under these conditions causes the heart to beat faster or slower in the same manner that it does after warming or cooling of the sino-auricular node. The general interpretation of such results is that the auriculo-ventricular node, though capable of originating beats in the same manner that the sino-auricular node does, is normally prevented from doing so by the faster pace at which the sino-auricular node sends out impulses.

Nodal Tissue.—Histological investigation of the two nodes described above shows that peculiar types of cells make up the fibrous structures which are characteristic of both these regions. As these cells are of the same character in the two regions, the inference is that they are the structures which, above all others in the vertebrate heart, are adapted to respond to the so-called "inner stimulus." Although, as mentioned above, a strip of muscle, cut from any part of the vertebrate heart, can beat rhythmically in the right nutrient solution, this does not prove that heart muscle is normally the originator of beats. The fact that the heart of the chick embryo beats before any nervous structures have grown into it has been regarded as evidence in favor of the myogenic as contrasted with the neurogenic theory of the heart beat. It does not, however, disprove the theory that heart beats originate normally in nodal tissue of the fully developed heart. Though all parts of the heart muscle are rhythmically excitable, nodal tissues are so especially sensitive and so comparatively rapid in rate of response that they tend to initiate all beats of the intact heart. This is the view developed by modern research upon the vertebrate heart. If it is correct, then the long debate on the neurogenic vs. the myogenic theory of the heart beat resolves itself into a question of whether nodal tissue is more closely related, in accordance with its embryological development, to nervous or to muscular structures. This question awaits further morphological investigation.

Origin and Conduction of the Beat of the Invertebrate Heart.—Among the invertebrates, few species have been investigated with regard to the origin and conduction of the heart beat. Carlson studied the heart of the *Limulus* or horseshoe crab. The heart of this animal is a simple, tubular structure bearing upon its surface a network of nerve fibers and ganglia so situated that they can be dissected off from the heart without injuring the muscular structures. When they are so removed, the heart ceases to beat. Indeed, when the ganglion cells of the so-called median cord of these structures are destroyed or excised the heart ceases to beat. These facts indicate that in this particular organism heart beats originate in the ganglion cells. Carlson also showed that the conduction of excitation which insures orderly contraction during the cardiac cycle is dependent upon the nervous structures. The heart musculature, quiescent after removal of the nervous structures, will contract when excited by artificial stimuli, such as induction currents, but it does not originate rhythmic beats and it does not conduct excitation in a normal manner. The neurogenic theory of the heart beat is thus applicable in this particular case. To what extent this theory applies to the hearts of all invertebrates is not known. Results as obtained with the *Limulus* heart have no bearing upon the theory of the origin of the beat of the vertebrate heart because the physiological properties of the *Limulus* cardiac muscle are distinctly different from those of vertebrate cardiac muscle.

The Refractory Period of the Heart.—As explained above, any living tissue shows a period just after it has been excited during which it is not excitable. This is its refractory period. This period is surprisingly long in vertebrate cardiac muscle as compared with the refractory periods of other muscles or of nerves. The tracing shown in Fig. 65 is sufficient to indicate that, throughout practically the whole period of its systole, the ventricle is unresponsive to an electrical stimulus applied directly to it. Only when a stimulus reaches the heart near the end of its period of systole or during its diastole does it arouse a response in the cardiac muscle. It is also noticeable that the latent period between the application of the stimulus and the corresponding response of the muscle is much longer when the stimulus is given during systole or early stages of diastole than it is when the stimulus is given later in the cardiac cycle especially in the rest period. The record also shows that after the response to the artificial stimulus, the heart shows a prolonged period of rest, the *compensatory pause* before it resumes automatic beats. This pause lasts until the next succeeding wave of excitation, coming from the pace setter, reaches the heart musculature in the observed region. The long refractory period of the heart explains one peculiar property of vertebrate cardiac muscle. This property is its rhythmic rather than tetanic response to tetanizing stimuli. It is incapable of producing a sustained rigid contraction or tetanus such as other types of muscles give. In this respect it is unlike invertebrate cardiac muscle which can be tetanized and does not show a long refractory period.

Extra Systoles.—Responses of the heart to artificial stimuli produce what are called extra systoles. In these the regular rhythm of the heart beat is disturbed. Under pathological conditions extra systoles are sometimes observed either in the auricle or in the ventricle of the human heart. Their occurrence is best recognized by observation of the electrocardiogram. Their occurrence is apparently due to the origin of beats in parts of the heart that do not normally originate them. Although the entire heart musculature is usually kept at work at a rate determined by the "pace setter," certain portions may break away from this controlling influence and start upon a course of independent discharge of rhythmic impulses. The origin of these extra systoles is probably in the nodal tissue between auricles and ventricles but this point is difficult to determine. Peculiar and partly uncoordinated action of the auricles and ventricles may also arise through a failure of the bundle of His to conduct excitation normally. Infection and other diseased conditions may bring about this condition. It results in a larger number of auricular than of ventricular systoles. In some cases, the auricles beat two to three times to one beat of the ventricles. This condition is not to be confused with the occurrence of extra systoles in the auricles for these differences in the rhythms of the heart chambers can best be

explained on the basis of imperfect transmission through the auriculoventricular node. This condition is therefore called *heart-block*.

The Arterial Pulse Wave.—The pulse in a blood vessel is the swelling of its walls due to the volume of blood forced out by each ventricular systole. The pulse is possible because of the elasticity of vascular tissue. If blood vessels were rigid, each heart systole would force as much blood out of any blood vessel as was forced into it during the time of the systole. This would mean, of course, great fluctuations in pressure and enormous strains upon the whole system. But as blood vessels are not rigid, the swelling of the arteries relieves much of this strain and enables them to give space for a part of the blood issuing from the heart during systole. This swelling proceeds from the aorta along the entire arterial system as a compression wave, that is, the entire arterial system does not swell at once but shows the pulse at different times at different distances from the heart. The progress of this pulse wave can be seen in an exposed artery. It can also be recognized by simultaneously feeling the pulse at different points. If, for example, one feels the carotid pulse with the fingers of one hand and the radial pulse with those of the other, the different timing of the two pulses is easily recognized. The rate at which the pulse wave spreads over the arterial system can be measured upon a graphic record of pulses traced simultaneously for two arteries at known distances from the heart. Such records show that the pulse wave in adult human beings travels at a rate of from 6 to 9 meters per second. The rate is different in different parts of the arterial system and also tends to increase with age owing to the progressive decrease in arterial elasticity. In arterial sclerosis, the rate may be as high as 13 meters per second. The progress of the pulse wave is independent of the rate of the blood flow. The pulse is a pressure wave and travels faster than does the current of the blood, just as a surface wave or ripple may progress faster down a stream of water than its current moves.

The Graphic Record of the Pulse. The Sphygmogram.—The rising and falling of the wall of an artery as each pulse wave passes it may be transmitted to a suitable lever device which graphically traces the pulse upon a moving surface. An instrument (Fig. 152) designed for this use is called a sphygmograph (from the Greek for pulse writer). The graphic record obtained is a sphygmogram. (Fig. 153.) The tracing shows an upstroke and a downstroke for each pulse wave. The first is called the *anacrotic* and the second the *catacrotic* limb. The first is produced by ventricular systole. Smaller secondary waves normally appear on the catacrotic limb. The chief of these is called the *dicrotic notch*. The pressure wave which produces the dicrotic notch is sometimes so pronounced that it can be detected by merely feeling the pulse. The cause of the dicrotic notch is the closure of the semilunar valves. This is shown by the fact that the notch disappears from the sphygmogram of an animal

with its semilunar valves hooked back and also by the fact that the notch is abnormal in the sphygmogram of a person whose aortic valves are insufficient. (Fig. 154.) The dicrotic notch is preceded by a negative wave as shown in Fig. 153. As the ventricle begins to relax, blood under maximal pressure in the distended aorta tends to rush back into the ventricle. This causes a backward surge in the arterial system and

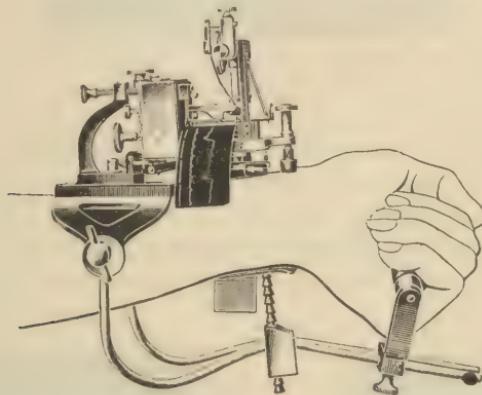


FIG. 152.—Sphygmograph, wrist form. (*From the catalogue of A. H. Thomas Co.*).

thus produces the negative wave. But concurrent with this event, the semilunar valves close suddenly. This closure, accompanied as it is by the high pressure still prevailing in the distended, elastic aorta, starts a positive wave. This produces the dicrotic notch. (D in Fig. 153.) Other smaller waves, some preceding the dicrotic (predicrotic), some

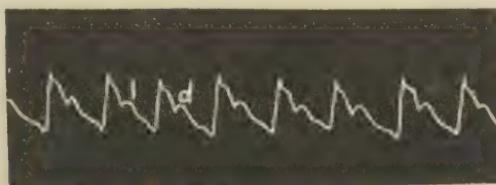


FIG. 153.—Sphygmogram. The dicrotic notch is shown at d.

following it (postdicrotic) are more difficult to explain. The explanation commonly given is that they are reflected waves thrown back into the arterial system when the main pulse wave reaches the points where the larger arteries divide into smaller branches. In agreement with this view, the various small waves vary on the sphygmograms of different arteries. They vary in their timing and in their shape. The dicrotic wave, on the contrary, always occurs at the same time relative to the main pulse wave. Secondary waves on the anacrotic limb of the sphygmogram occur rarely in pathological conditions. They have not been explained

satisfactorily. According to one view, they are caused by an abnormally rapid reflection of the main pulse wave from the peripheral branchings of the arterial system. This condition tends to occur in hardened arteries.



FIG. 154.—Sphygmogram of a case of valvular insufficiency, dicrotic notch indistinct and variable.

The sphygmogram varies with the blood pressure. A so-called soft pulse, obtained in cases of low blood pressure shows the general form represented by the tracing A in Fig. 155. The so-called hard pulse, accompanying high blood pressure gives a tracing such as that shown in B. Sphygmograms are used in a clinical study of the circulation and in diagnosis.

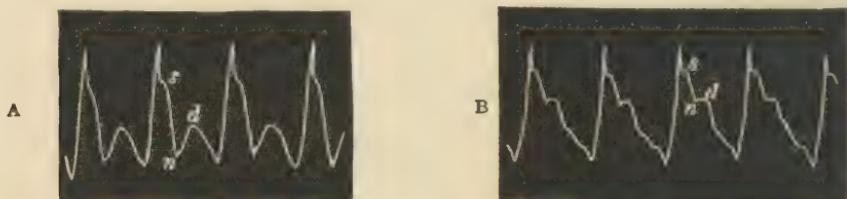


FIG. 155.—Sphygmograms showing variations in the character of the pulse. A, soft pulse. B, hard pulse. (From Burton-Opitz, "Text Book of Physiology," W. B. Saunders Co.).

The Venous Pulse.—As mentioned above, no pulse occurs in the capillaries because of the high resistance there offered to the circulation. But in the veins, especially in the large ones, a distinct pulse occurs. It is due, however, to causes that are different from those producing the arterial pulse. It is weaker than the arterial pulse, so that it is best recorded by a more sensitive registering device than that used upon an artery. One of the several waves of which each main venous pulse wave is composed, is due to the pressure developed by auricular systole and exerted upon the blood in the veins. Another of these waves is probably due to the protrusion of the auriculo-ventricular valves into the auricle. Still another of the waves is probably the result of the flow of blood from the auricles into the ventricles during the diastole of the latter. The study of the venous pulse helps to an understanding of the normal and pathological behavior of the auricles and the veins just as the arterial pulse aids in the examination of conditions in the ventricles and arteries.

The Nervous Regulation of the Circulation.—Conditions in the entire circulatory system are under constant regulation chiefly by the nervous

system. This regulation determines the rate and force of the heart beat, the size and tone of arteries, and to some extent the behavior of capillaries and venules. The regulation controls the changes of the general level of blood pressure in the circulation as a whole and the relative pressures in specific parts. It also controls the relative supply of blood furnished to each organ or system of organs from minute to minute. This regulation is useful because the relative abundance of the supply of oxygen and nutrients required by any specific organ varies with its relative activity at any given time. Muscles, when hard at work, require a larger blood supply than when at rest. The digestive organs, the nervous system, the liver and other glandular structures similarly vary in their demands upon the blood. Moreover, the regulation of the temperature of the body requires that the relative blood flow through regions near the skin shall be so adjusted as to aid in regulating the relative loss of heat by radiation from the blood through the surface of the body. It will be convenient to describe separately the nervous regulation of the different parts of the circulation.

The Nervous Regulation of the Heart.—The action of nervous impulses, reaching the heart by way of the vagus nerve, was discussed in connection with the subject of inhibition (p. 286). The constant activity of vagus inhibition, keeping a steady "brake" upon the heart, was explained in connection with reflexes acting through the cardio-inhibitory center (p. 289). The especially significant case of reflexes through the depressor nerve, the cardio-inhibitory center and the vagus was detailed. Inhibitory influences probably take effect upon the pace setter at the sino-auricular node. Endings of vagus nerve fibers appear to occur at this node and electrical stimulation through electrodes applied to the frog heart at the sino-auricular junction can inhibit the heart. Further evidence favors the idea that the vagus takes effect upon the pace setter. The ventricle is not inhibited by stimulation of the vagus unless the auricles are also inhibited although a slight decrease in the force of auricular beats has been observed during mild stimulation of the vagus without a simultaneous effect upon the ventricle. It has also been shown that with a clamp on the auriculo-ventricular node, causing the ventricle to assume an independent rate, stimulation of the vagus may cause complete stoppage of the auricle without affecting the ventricle.

The activity of the heart may be increased by means of nerve impulses. These reach the heart by way of the cardiac sympathetic fibers. They are called the *accelerator nerves*. After they are cut, stimulation of their peripheral ends may produce an increase in the rate of the heart beat with either an unchanged or a diminished force of the individual contractions or it may cause an increase in the force of the contractions with or without an increase in the rate. These differences in the effects are not completely explained, but have been assumed by some writers to be due

to the existence of two kinds of fibers in the cardiac sympathetic nerves. According to this theory, one kind is the accelerator which affects the rate of the heart and the other kind is the augmentor which affects the force of contractions. If these two types of fibers exist, the different effects of sympathetic stimulation are determined by the relative number of each kind of fibers that are artificially excited. The theory seems plausible because, in certain animals, stimulation of the cardiac sympathetics on the left side is more apt to cause augmentation without acceleration of the heart than is stimulation on the right side.

Tonic (sustained) activity of cardiac acceleration is demonstrated by experiments comparable to those which show tonic activity of cardiac inhibition. These experiments consist in cutting all the cardiac sympathetic fibers on both sides of the body. If, during this operation, the heart rate is appropriately recorded, a slowing is seen to follow the cutting of these nerves. Accelerator fibers are thus shown to convey a steady stream of excitatory impulses to the heart. This fact suggests reflex excitation as the source of the constant stream of impulses. But the actual occurrence of reflex acceleration of the heart is difficult to demonstrate. Stimulation of sensory nerves may cause, it is true, a hastening of the heart; but this is not a proof that a reflex effect is exerted through the accelerator fibers. It might readily be the result of an interference with the flow of impulses from the cardio-inhibitory center through the vagus nerves. As explained above, this "inhibition of inhibition" occurs when all the accelerator fibers to the heart are cut. It would seem as though a reflex hastening of the heart acting through the accelerator nerves could be demonstrated after both vagus nerves were cut; but all attempts to do this have failed, probably because the heart, released from vagus inhibition, assumes a rate so rapid that further increases through reflex excitation cannot be obtained. Hooker made this explanation plausible by showing that when the heart was slightly inhibited by mild electrical stimulation of the peripheral ends of the two severed vagi, acceleration of the heart could be demonstrated during excitation of certain afferent nerves. This experiment furnishes an instance of reflex heart excitation occurring through the accelerator nerves. Further evidence was obtained by Hering who showed that the heart acceleration which normally follows muscular exertion does not occur in an animal whose accelerator nerves have been cut.

One instance of reflex acceleration of the heart as reported by Bainbridge is of the nature of an automatic regulation. The stimulus is a distending pressure in the vena cava, a pressure such as develops from exceptional fullness of the large veins. The response is a hastening of the heart thus tending to relieve venous distention. Bainbridge believed it to be due, in part, to diminished vagus inhibition, in part, to reflex

acceleration. The nervous path, the arc of this reflex, includes sensory fibers in the vagus with receptors in the heart.

Thus two reflex mechanisms, the inhibitory and the accelerating, act upon the heart. Either or both of them can modify, at any given moment, the rhythmic activity originating in the heart. These two modifiers are antagonistic. Hunt and others have shown that when varied strengths of electrical excitation are applied simultaneously to the vagus and the cardiac sympathetics, the resulting heart rate is determined by the relative strengths of the two stimuli. In other words, the final effect on the heart is the "algebraic sum" of the excitations of inhibitory and accelerating fibers. This dual control of the heart might seem an unnecessary duplication of mechanisms. It furnishes two means for reflex slowing of the heart: Excitation through the cardio-inhibitory center and a diminution of impulses through the accelerator nerve. It also affords two different ways of accelerating the heart: Diminution of inhibitory reflexes and increase of accelerating ones. This duality of control, however, is probably a distinct advantage, in that it makes the heart delicately responsive to the changing conditions in the body and its environment.

The Nervous Regulation of Arteries.—The action of vasoconstrictor nerves was described above. These nerves belong to the sympathetic system, the thoracico-lumbar autonomies, and run through the spinal and cranial nerves to all parts of the body. They affect the tonus of the muscles in the walls of all arteries and arterioles, but rapid and significant changes of this tonus are produced more especially in the arterioles. This sympathetic vasoconstriction is the result of reflex excitation. Tonic activity of the vasoconstrictor nervous mechanism was first demonstrated by Claude Bernard (1851). The famous experiment by which he demonstrated this was performed upon an albino rabbit. The ears of this animal are sufficiently thin and translucent to enable the arteries to be clearly seen by transmitted light. The experiment consisted in cutting the cervical sympathetic nerves. This results in visible dilatation of the ear arteries and a marked reddening of the ear, because cutting of these nerves interrupts the flow of impulses which maintains arterial tonus. Artificial electrical stimulation of the peripheral ends of the severed nerves causes a visible constriction of the arteries with accompanying paleness of the rabbit's ear. The vasoconstrictor reflex center for maintenance of arterial tone was described in connection with the medulla. Even after the medulla is completely separated from the spinal cord, arterial tone is not entirely abolished. This was shown by Goltz, working with frogs and dogs. It indicates that reflex vasoconstriction may employ nervous arcs centered in the spinal cord and therefore shorter than those involving the main vasoconstrictor center in the medulla. Vasoconstriction after the spinal cord is divided in the cervical

region has been obtained by several observers upon stimulation of sensory nerves

Several types of methods have been adapted for the modern study of vasomotor nerves. One of these methods is the measurement of blood pressure changes in the arteries in response to nerve excitation and other conditions. Another method depends upon the measurement of volume

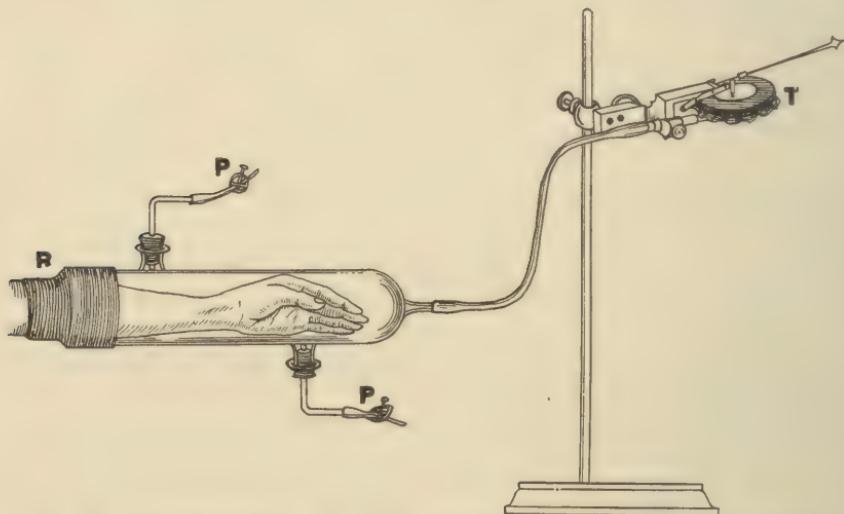


FIG. 156.—Plethysmograph. *R*, rubber cuff which makes air-tight connection between the glass part of the instrument and the arm. *P* and *P*, pinch cocks which are closed after the arm and the instrument are adjusted for a record. *T*, delicate tambour consisting of a metal box covered by a thin rubber membrane upon which rests the lever whose point traces the record. A side tube opening in the tambour is connected to the glass chamber by rubber tubing.

changes in specific parts of the body, such as the limbs, or in single organs such as the heart, the kidney or the spleen. The instrument used to obtain graphic records of these volume changes is called a **plethysmograph**. (Fig. 156.) Dilatation of arteries in the part of the body under observa-

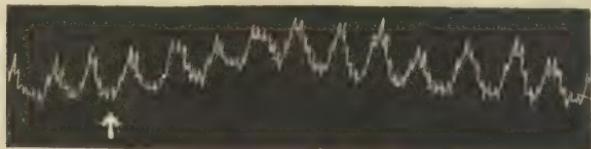


FIG. 157.—Plethysmogram. The smallest waves are due to individual heart beats, the larger ones to respiratory effects. The largest wave, lasting during eight respirations and beginning at the point marked by an arrow is due to a vasomotor reaction.

tion increases the volume of the part. Arterial constriction decreases the volume. The volume may also change in a passive way as a result of marked changes in other parts of the body. Such volume changes are recorded in the **plethysmogram**. (Fig. 157.)

The *afferent nerve fibers* for vasomotor reflexes may be divided into those which cause vasoconstriction, the **pressor nerves**, and those which cause vasodilatation, the **depressor nerves**. Pressor nerve fibers are widely distributed. They occur in the majority, perhaps all, of the spinal and cranial nerves which contain afferent fibers from the skin region. Probably all the nociceptive (pain) nerves can elicit vasoconstrictor reflexes, and many other afferent fibers also produce this effect. The multiplicity of these reflexes shows that the vasoconstrictor center is the converging point of very many afferent paths. Depressor fibers have not been clearly demonstrated to occur so generally. Their presence may be obscured to experimental detection, in many cases, by the occurrence of pressor fibers in the same nerve trunks. This is indicated by the fact that electrical stimulation of the central stump of a given nerve may cause vasodilatation during deep anaesthesia although, the same procedure produces vasoconstriction during lighter anaesthesia. This indicates that pressor influences tend to predominate, under some circumstances, over the depressor ones. In some cases, depressor nerves can be clearly identified because they are present in nerve trunks which do not contain any pressor fibers. The most notable instance is that of the depressor nerve of the heart. Its stimulation produces vascular dilatation. Other instances are found in the case of afferent nerves from some of the sexual sense receptors. Their stimulation produces the arterial dilatation which is incident to the engorgement of erectile tissues with blood.

There apparently are two ways in which vascular dilatation may be effected by reflex control. One of these is by decreasing the tonic activity of the vasoconstrictor center, that is, inhibition in the central nervous system. This explanation is commonly given for the effect of stimulation of the depressor nerve of the heart. It is also applied to the vasodilatation in the skin regions during heating of the skin.

The other explanation regards vasodilatation as produced reflexly through the *vasodilator nerves*. These were described above in connection with inhibition. Comparatively few regions of the body have been shown, unquestionably, to have their arteries supplied with true vasodilator fibers, that is, fibers which, when stimulated, cause inhibition of arterial tonus. The vasodilator nerves of the salivary glands were discovered by Claude Bernard. Those of the penis were discovered by Eckhardt. Their action has been extensively investigated. Good evidence is also available to show the existence of such fibers for parts of the tongue and pharynx, for the mucous membranes of the lips, gums and palate, for the nostrils and for the skin of the cheeks. Vasodilatation of the arteries of the limbs and main parts of the body is obtained as a result of direct nerve excitation only under certain circumstances.

Further investigation will be required to determine the extent to which vasodilator nerves influence the main arterial system.

Vasodilatation, mediated reflexly through vasodilator nerves, constitutes presumptive evidence for the existence of a *vasodilator center*, corresponding to the vasoconstrictor center. But no definitely localized part of the central nervous system has been shown to be a requisite part of numerous different reflex arcs for vasodilatation. In the case of one vasodilator reflex, that involved in erection of the penis, Eckhardt showed that the reflex arc was centered in the dog in the lower part of the spinal cord. Stimulation of the glans caused normal erection after the spinal cord was severed in the lower thoracic region. It would seem, therefore, that vasodilator reflexes are centered in different parts of the nervous system rather than in one restricted region.

Nervous Regulation of Capillaries and Venules.—During the sixty years following Claude Bernard's discovery of vasoconstrictor effects, the majority of physiologists believed that nervous regulation of blood vessels was exerted solely upon arteries and, indeed, chiefly upon arterioles. Newer researches, conducted by Bayliss, Dale, Hooker and others, have produced evidence to show that the capillaries and venules are also nervously regulated to a physiologically significant extent. This idea has been somewhat difficult to accept because of the structure of the capillaries. No muscle cells are present in their walls which are merely a single layer or mosaic of simple, thin endothelial cells. The walls of venules, though more complex than those of capillaries, are also devoid of muscle. They contain considerable connective tissue in addition to endothelial cells. Both capillaries and venules possess nerve plexuses similar to those of arteries. Presumably, these plexuses have motor effects. Stimulation of certain sympathetic nerves does, indeed, cause constriction of capillaries. This can be seen by microscopic observation of thin tissues, such as the nictitating membrane of the frog eye, the tip of the ear of the cat and the thin tissues of the omentum, during electrical stimulation of the proper sympathetic nerve. The capillaries grow smaller in diameter according to actual measurement or they entirely disappear from view under low magnification. (Fig. 158.) The same result can also be obtained by injection of the right strength of adrenine solution (1-50,000). According to the generally accepted view, adrenine causes excitation of sympathetic nerve endings. Its action in this instance thus constitutes presumptive evidence of the existence of a sympathetic nerve mechanism for capillary constriction.

Tonic activity of sympathetic control of the capillaries is postulated. No experiments have satisfactorily demonstrated, however, that cutting of the sympathetic nerves decreases the tone, that is, causes dilatation, of capillaries. The failure to demonstrate this is due, chiefly, to the difficulty involved in detecting small increases in the size of capillaries.

Dilator effects, corresponding to vasodilatation of arteries, have not been observed in capillaries as a result of nerve stimulation. Changes of individual capillaries, independent of neighboring ones, have been observed. This indicates that capillaries can determine the relative blood supply to each small portion of an organ.

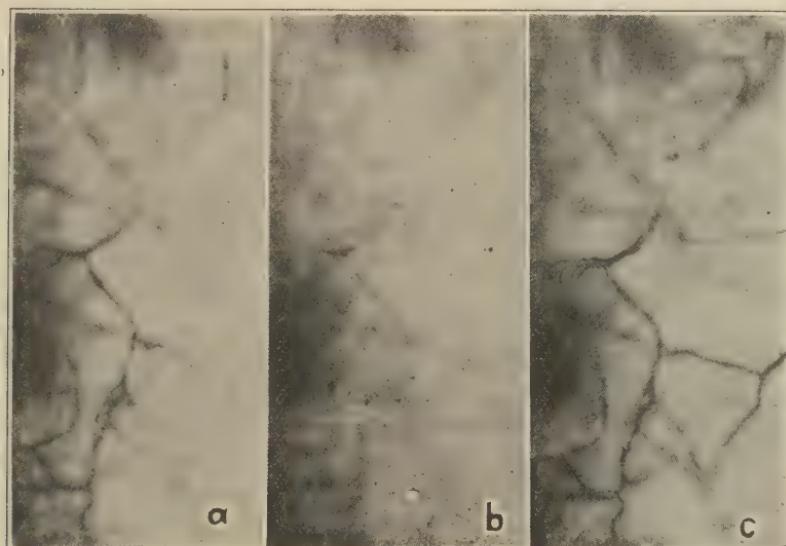


FIG. 158.—Three microphotographs of the same area of the ear of a cat to show capillary constriction. *a*, at the beginning of the experiment. *b*, during stimulation of sympathetic nerves. *c*, after stimulation ceased. (From Hooker, *Physiol. Rev.*, vol. 1).

Venules also constrict in a manner similar to the behavior of capillaries but apparently do not occlude the lumen completely as do capillaries.

The mechanism of constriction of vessels devoid of muscular tissue can only be surmised as yet. Most workers agree that it is due to changes in the shape of the endothelial cells. This change might be imagined as an amoeboid motion; but, as Hooker points out, such a change in shape would necessitate a change in the length of the capillary to compensate for the change in diameter. Changes in length are not observed in constricting or dilating capillaries. A more acceptable view, favored by several investigators, is that the endothelial cells can swell by an intake of fluid and thus become sufficiently turgid to occlude the lumen of the capillary or can become unusually depleted in their fluid contents and thus dilate the capillary.

Nervous Regulation of the Veins.—The walls of veins contain muscular tissue which appears to maintain a tonic constriction. The veins are provided, to some extent at least, with sympathetic nerve connections. The presence of these structures suggests that the veins might be under nervous regulation. Under most conditions of experimental observa-

tion, however, veins do not show evidence of active changes in tone such as arteries so clearly display. In only a few instances has experiment demonstrated changes in venous tone as a result of nerve excitation. Even in these few instances, objection to the evidence or to its interpretation as a vasomotor effect has been raised.

It is therefore doubtful to what extent a nervous control is exerted upon veins. If it does occur it plays a rôle which is inconspicuous, compared to the nervous regulation of other parts of the circulatory system.

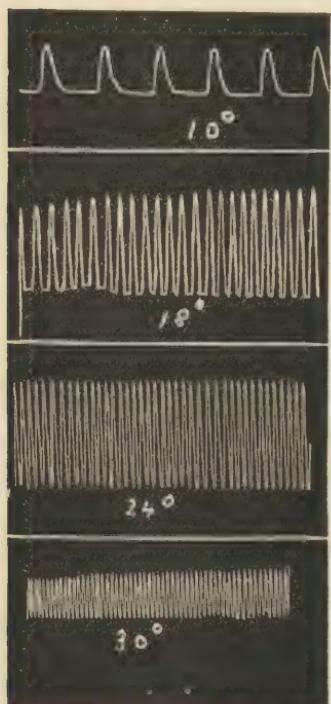


FIG. 159.—Effect of temperature on the frog heart. (From Howell's "Text Book of Physiology," W. B. Saunders Co.).

Effects of the Blood upon the Organs of Circulation—Chemical Regulation.—The temperature of the blood exerts marked effects upon the heart. The hastening of the heart rate is strikingly shown with rise of temperature of the medium surrounding the excised heart of a cold-blooded animal. (Fig. 159.) Between the temperature 5°C. and 30°C. the frog heart in Ringer solution shows steady increase in rate and in extent of the individual beats. The optimum temperature is about 30°C. At higher temperatures the frog heart shows decrease in rate and force and falls into an irregular rhythm or fluttering action. At about 38°C., beating is permanently abolished. The mammalian heart is active within a smaller range of temperatures, from about 17°C. to about 45°C. Within the range 28° to 39°C., the excised heart of a dog beats at rates which increase and decrease

with rise and fall of the temperature of the blood surrounding it. In the case of hibernating animals, the lowered temperatures that prevail during hibernation are accompanied by distinctly lessened heart rates.

The marked effects which are produced by changes in the concentration of potassium, calcium or hydrogen ions, show that changes in the nutritive medium of the heart can alter its behavior. These changes do not occur normally to an extent that is comparable to conditions produced during experimental manipulations. The composition of the normal blood is regulated accurately and sensitively as regards the concentration of these particular ions.

Certain internal secretions probably function in chemically regulating the circulation of the normal animal. Thyroid glands, when fed, cause distinct effects upon the heart, producing a faster pulse and higher blood

pressure. Although the active thyroid principle, thyroxine, has not been detected in blood, it probably is normally present there. This is indicated by the slower heart rate and other changes which result from partial removal of excessively active thyroid glands of human beings or from complete removal of normal thyroids of animals. Adrenine, secreted into the blood from the adrenal glands, causes augmentation of the heart under certain circumstances, produces dilatation of arteries when certain low concentrations of adrenine are present in the blood and causes very marked vasoconstriction so as to greatly raise blood pressure when higher concentrations of adrenine are present in the blood. This reaction probably serves to insure the necessary augmentation of the circulation during certain emergencies. An unidentified substance, obtained by extraction of the pituitary gland also causes arterial constriction. Its effective presence in the blood stream of the normal animal has not been demonstrated. The action of thyroxine, of adrenine, the pituitary substance and other internal secretions will be described in more detail in Chap. XXV. The CO₂ content of the blood affects the heart rate. Increase of CO₂ causes a faster rate. Chemical products of body tissues are able, then, to exert some regulatory action upon the circulation but the most powerful of these chemical agencies, adrenine, is secreted into the blood under the influence of nerve stimuli to the adrenal glands. Whether or not it may be secreted irrespective of this nervous control cannot be stated at present. Carbon dioxide production is largely determined by muscular activity which, in turn, is determined by nerve stimuli. It thus appears that the nervous system is, in a sense, in control of some at least of the chemical regulation of the circulation.

Many drugs, especially the so-called alkaloids (nitrogen-containing bases of vegetable origin) exert marked effects upon the circulation. Muscarin, an alkaloid from the fly-fungus, *amanita muscaria*, slows or stops the heart and produces this effect even upon an excised heart. Atropine, an alkaloid of belladonna from the deadly nightshade, releases the muscarin-poisoned heart to a faster rate. Inasmuch as atropine is known to cause paralysis of endings of certain nerves, its effect upon the heart is explained as being due to paralysis of vagus nerve endings which were previously excited by muscarine. Digitalis, a drug prepared from the leaves of foxglove, contains several glucosides, including digitalin and digitoxin. Digitalis, in suitable dosage has a slowing and steadyng influence upon the circulation. It causes a decrease in the rate with increase in the force of the heart beat and a distinct vasoconstriction. Because of these tonic effects on the circulation, digitalis has been extensively used in the treatment of certain heart diseases. The study of the action of these and numerous other drugs upon the heart and arteries constitutes one of the large aspects of experimental pharmacology.

Chemical effects are also exerted upon the capillaries. These effects consist of dilatation in all cases that have been observed. The capillaries are normally in a state of partial constriction owing to influences of which nerve excitation is probably one. Decrease of this tonus can occur presumably as a result of diminished nerve excitation; but this effect is not so clearly demonstrated as are those which show dilatation of the capillaries by chemical means. Of substances producing this effect, histamine has assumed an especial significance. It is produced by certain kinds of oxidation of the amino-acid, histidine. Histamine is probably liberated into the blood from dying tissues of wounds. Typical symptoms of wound shock, such as follow the production of massive wounds, can be produced in experimental animals by injection of histamine. Histamine has been identified in the disintegrating tissues of wounds. Bayliss, Cannon and others have extensively investigated wound shock. Its chief symptom is a marked and dangerous fall of blood pressure. This appears to be due to a great dilatation of capillaries with accompanying stagnation of blood in the tissues. This diminishes the volume of blood in the arteries and causes fall of blood pressure. Histamine is known to dilate capillaries. Whether its production is the sole cause of wound shock or whether the production of numerous substances, of which histamine is an example, causes shock, is not known. The use of gum arabic solutions and of blood transfusions for combating wound shock was discussed previously (p. 151).

Adjustment of the Circulation.—The blood supply tends to be adjusted to the varying needs of the body as a whole and of its separate organs. The blood supply is increased in organs that are especially active. Increased activity in a small organ such as the salivary gland does not require a marked readjustment of the circulation. It merely involves a dilatation of the arteries of the gland itself. But when a marked increase in the activity of a large organ or group of organs occurs, very noticeable readjustments of the circulation are required. Active digestion or excessive muscular exertion, for example, require increased blood supplies to so large a part of the body that one of two sorts of reactions must occur. Either the blood supply to other regions must be restricted or else the output of blood from the heart must be increased. Either or both of these reactions may occur. In heavy muscular exercise the heart rate is especially increased. Conditions other than increased activity of an organ may result in an increased blood supply to it. The best instance of this is exposure of the skin to high temperatures. This causes marked dilatation of the arterioles leading to the skin. The resulting increased blood flow near the surface of the body aids in the radiation of heat and consequent regulation of body temperature. Cold also affects the skin circulation. Slight chilling tends to cause constriction of the skin arterioles and a resulting paleness of the skin. Certain degrees of cooling

may cause capillary dilatation without a corresponding increase in the rate of blood flow through the skin. A tendency to stagnation of blood in the skin area is thus produced and results in considerable deoxygenation of the blood. This causes the blue appearance of the chilled skin.

The blood supply to the brain is subject to regulation. Normal sleep is accompanied by a decreased blood flow through the brain. Some evidence for this has been obtained from observations upon infants. The spot at the top of the skull, where the cranial bones are not sutured in the infant, sinks in slightly during sleep. This indicates decreased volume of the cranial contents and is explained as due to a decreased blood supply to the brain during sleep. Certain of the cranial arteries have been

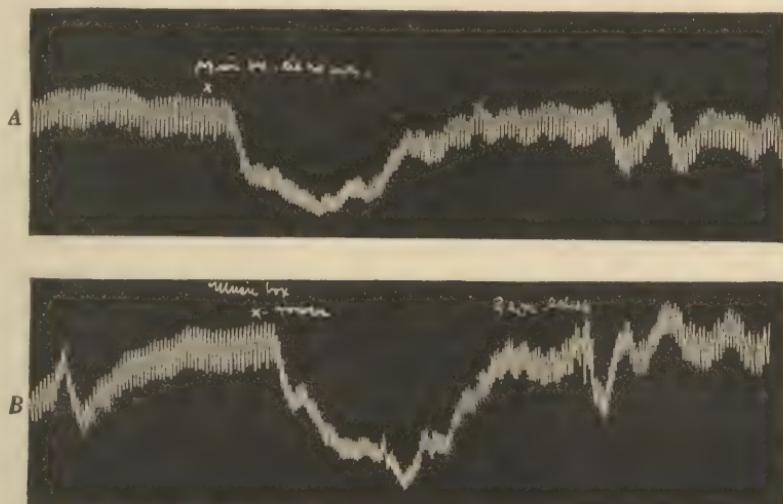


FIG. 160.—Plethysmograms taken during sleep. *A*, showing vasoconstriction at the sound of a music box which did not waken the sleeper. *B*, the same with a brief period of wakefulness, accompanied by a comparatively great vasoconstriction, soon followed by sleep with vasodilatation. (*From Howell's "Text Book of Physiology," W. B. Saunders Co.*).

observed, in cases of skull fractures, to constrict during sleep. This decreased blood supply to the brain is accompanied by an increased blood flow through the muscles and skin regions. This fact has been established by plethysmograph records (Fig. 160) which show a swelling of the arm or leg during sleep. The swelling begins at the moment of falling asleep and tends to persist until the time of wakening. The blood supply to the brain is also regulated in accordance with mental activity and emotional states. Constriction of the arterioles of the arm, with decrease in its volume, can frequently be observed in plethysmograms recorded during mental efforts. This reaction is interpreted as signifying an increased blood flow to the brain. Emotional excitement may yield similar records. The emotions can produce very profound effects

upon the circulation. These are exerted upon the heart rate as well as upon vascular constriction.

The Circulation of Lymph.—The main cause of lymph flow is the difference in pressure between the lymph vessels in the tissues where lymph is produced and the vein into which the thoracic duct empties the lymph. A valve at the opening of the duct prevents back flow of blood into the lymph system but permits lymph flow into the veins as long as the venous pressure is less than that in the lymph vessels. The actual amount of pressure in the lymph spaces of tissues is not known but it must be considerable as is indicated by the turgidity which all normal animal organs show. Inasmuch as lymph is produced from blood, the pressure within blood capillaries is partly responsible for lymph pressure. The heart beat is thus an indirect but nevertheless prime cause of lymph flow. This is true at least in mammals. In some animals, for example, frogs, lymph hearts actually pump the lymph. Even in mammals, part of the thoracic duct is capable of contracting, but the extent to which its activities influence lymph movement is doubtful.

Certain accessory factors aid in propelling the lymph. The chief of these is muscular contraction. Peristalsis of intestinal muscles helps to force the chyle into and therefore through the lacteals. Contractions of skeletal muscles tend to propel lymph along the vessels of the limbs and the trunk. This is experimentally proved by observations on the flow of lymph from a fistula in the thoracic duct. Such observations show that any muscular movements tend to increase the outflow of lymph. Massage has a similar effect. Pressures exerted by active muscles or by any other means can move lymph in only one direction, namely, toward the thoracic duct. This is due to the arrangement of valves in the vessels of the lymph system. Deficient movement of lymph may cause a temporary oedema. A well-known case is that of the swelling of the lower parts of the legs of horses after they have stood for an unduly long time without having any exercise. Stagnation of lymph causes this swelling which rapidly disappears during exercise. Another accessory factor for lymph movement is breathing. Each inspiratory movement of the thorax tends to suck lymph into the thoracic duct and from thence into the veins. Because of the terminal valve of the duct no backward movements of lymph are produced by expiration.

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CHAPTER XX

RESPIRATION

Respiration in its widest meaning is sometimes defined as the gaseous exchange between an organism and its environment. Its essential features are the intake of oxygen and the output of carbon dioxide. Small organisms take oxygen directly from the air or from the watery medium surrounding them into all parts of their structures. They also give off carbon dioxide from all their parts directly into the surrounding medium. They can do this successfully because they are small enough to have every cell in direct or nearly direct contact with the environment, so that diffusion of gases can occur into and out of every cell. Larger forms require the aid of respiratory and circulatory systems to permit satisfactory gaseous exchange and distribution.

The gaseous exchange between the blood or other circulatory medium and the air or the surrounding water constitutes what is called *external respiration*. The gaseous exchange between the blood or other circulating fluid and the active cells of the organism constitutes what is called *internal respiration*.

Respiration in its truest sense is a part of metabolism for it marks the beginning and the end of oxidative processes. These are the chief means for liberation of the energy transformed in living matter. Life cannot be maintained very long without renewal of its oxygen supply and removal of carbon dioxide, the chief product of physiological oxidations. The failure of nearly all living structures to maintain any actual store of available oxygen is an established fact. Continuous respiration is therefore necessary to maintain oxidative metabolism and this is necessary for life.

History of Discovery of Oxidation.—An Englishman, Robert Hooke, was the first to demonstrate clearly the significance of respiration (1664). He showed that breathing movements are not requisite for life. A dog, with thorax opened and respiratory muscles cut, can be kept from asphyxiation by pumping air in and out of the lungs from a bellows or even by blowing a current of air through the lungs after they have been punctured. Hooke thus showed that breathing is important only in so far as it serves to expose the blood of the lungs to renewed supplies of air. Mayow (1674) first showed that something necessary for life was taken from the air. He thus gave a forecast of the future knowledge of oxygen requirements. He spoke of the requisite substance as “spiritus nitro-aereus,” but assumed it to be in particles, the nitro-aerial particles.

He clearly recognized that fire and living things are alike in that they both require something which they obtain from the air. Not until another century had passed was the true nature of oxidation made clear in terms of chemistry. This was done by the epoch-making researches of Lavoisier (1770). His analysis of air and expired air showed that respiration was essentially an oxidation process. Carbon dioxide had been previously discovered by Black (1755) who called it "fixed air." He had not appreciated its true relationship to oxidative processes as did Lavoisier. Priestley discovered oxygen at about the time that Lavoisier was doing his work. The latter gave the name oxygen to what Priestley called "dephlogisticated air." To Lavoisier belongs the credit for proving that oxygen is used and carbon dioxide produced by the same processes of life, namely vital oxidations.

Plant Respiration.—An apparent but not real exception to this rule of vital gas exchange is found in the case of green plants. While performing photosynthesis, they use more CO₂ than they give off and give off more O₂ than they take in. This was first shown by Priestley in his famous experiments in which mice were kept alive in an air-tight jar containing green plants exposed to sunshine. In the dark, however, the plant respiratory exchange is comparable to that of animals, in that O₂ is used and not evolved while CO₂ is given off and not used for synthesis of carbohydrate. At all times, plants require O₂ and produce CO₂ because of oxidation processes in their cells, even though the effect of this oxidation may be masked by that of photosynthesis. Higher plants, like complex animals, require structural arrangements for external respiration and for internal transport of gases. To a large extent, leaves are respiratory organs and circulating sap, though slowly moving, is a transporting agent.

The following account will deal more especially with the mammalian respiratory process because it has been studied more extensively than have the corresponding processes of other organisms.

Respiratory Movements—Breathing.—Some animals which lack a typical circulating system maintain a circulation of water through their bodies. An example of this is found in the case of sponges, which by means of ciliary motion cause a stream of water to flow through the body cavities. Although this arrangement secures a supply of dissolved oxygen for the cells, it is not strictly comparable to an external respiration because it merely provides for a direct gaseous exchange between the organism and its watery environment. Insects have a provision for a respiratory exchange which is more like a typical external respiration. They maintain a movement of air through ramifying tubes, the tracheae. Rhythical muscular movements maintain the movement of air in the tubes so that this action is comparable to the rhythmic breathing of vertebrates. The exchange of gases between the air of the trachea and

the active cells of the insect is a direct or nearly direct one. In a sense, then, insects do not have a true external respiration.

Vertebrates use gills or lungs as the chief organs of external respiration. The skin of some animals, for example frogs, serves as an additional place for gaseous exchange between the blood and the environment. In gills, the arrangement of structure is such that abundant blood flow through numerous capillaries gives opportunity for exchange of gase between the blood and the water in the gill spaces. A movement of water through the gills is maintained by muscular contractions that are rhythmic and are thus comparable to breathing movements of land forms.

Respiratory movements of air-breathing vertebrates are due to muscular contractions which alternately distend the elastic lungs and partially deflate them. The mechanism varies somewhat in different animals. That found in the frog and in the mammal will serve as examples.

In the frog, the relaxation of muscles in the floor of the mouth permits the mouth cavity to fill with air through the open external nares. Contraction of the same muscles, accompanied by muscular closure of the nares, forces air into the lungs with a bellows action. This movement constitutes inspiration. It is followed by expiration during which the muscles of the floor of the mouth relax and the nares open. A part of the air in the lungs then passes out because the lung tissues, stretched by the inspired air, tend to recoil when the pressure within them is reduced to that of the atmosphere. Elasticity of the lungs is shown by their complete collapse when air is admitted to the body cavity so that atmospheric pressure is exerted upon the exterior as well as the interior surface of the lungs. They can be again distended by blowing them up through the trachea.

In the mammal, the action of respiratory muscles such as those of the diaphragm and of the chest and abdominal walls produces changes in the capacity of the thorax. These are so generally described in connection with vertebrate anatomy that their complete description is unnecessary here. Certain matters connected with their action are not readily discernible from anatomical observation and must therefore be considered at this point.

Movement of the ribs during inspiration causes an enlargement of the thorax. The explanation of this is found in the way the ribs are hinged upon the vertebra. Each rib connects with its vertebra at two points. Therefore its movement is limited to a rotation around an axis passing through them. The direction of this axis is different in the case of different ribs. In the case of the first rib, the axis is directed across the vertebral column, that is, from right to left. Muscular contractions during inspiration pull the ribs in an anterior direction, thus enlarging the chest. Upon following the series of ribs along the thoracic region, there is found a progressive shifting in the direction of the axes of rotation. They tend

to become more nearly dorso-ventral. On this account, the more posterior ribs, when pulled by inspiratory muscles, not only move toward the anterior but are slightly twisted or rolled outwards. The position of the ribs at the end of respiratory movements is not at right angles to the spinal column. In man they slope downwards, in all animals, in a posterior direction. Because of this fact and because of the twisting motion imparted to some of the ribs their motion in response to contraction of inspiratory muscles slightly enlarges the thorax in all its diameters.

The numbers of muscles used in breathing varies greatly under different circumstances. In quiet breathing, **eupnoea**, fewer muscles are employed than in labored breathing, **dyspnoea**. During eupnoea in man, for example, inspiration is due to contraction of the diaphragm and a relatively small number of thoracic muscles, such as the external intercostals. Expiration during eupnoea is not due to muscular contractions so much as to passive factors: (1) The pull of gravity on the chest; (2) the elastic recoil of cartilages at the ventral ends of the ribs, cartilages which are put under slight tension during inspiration; (3) the movement of abdominal organs back to the position from which they are slightly displaced by the pressure put upon the abdomen during inspiration. This movement is slightly assisted in many cases by contractions of abdominal muscles. In any case, a movement of the diaphragm against the base of the lungs is produced. (4) The elastic recoil of the lungs which are fitted against the inner wall of the chest only by means of the atmospheric pressure exerted upon their interior. Although these forces are sufficient to cause quiet breathing movements, those of dyspnoea require the activity of a larger number of muscles. During labored inspiration, all chest and neck muscles capable of raising the ribs are thrown into contraction. The diaphragm also contracts to a much greater extent than in quiet breathing. During labored expiration, all chest and back muscles, which can pull down the ribs, are contracted. Muscles of the abdominal wall are used to a much greater extent than in quiet breathing. This larger group of muscles, used during dyspnoea, is so controlled, however, as to give the same rhythmic action that prevails in eupnoea. But in the convulsive breathing movements, which occur in the later stages of asphyxiation the respiratory muscles are not rhythmically controlled. Expiratory and inspiratory muscles may even contract at the same time. Indeed, the muscles of the entire trunk, and often those of the limbs as well, are thrown into convulsive contractions owing to insufficient oxygen supply.

Barring this extreme and unphysiological condition, however, nervous control secures perfect rhythmicity of the action of every respiratory muscle. This control regulates even those muscles which serve to produce mere accessory breathing movements such as the inspiratory distension of the nostrils in the horse, the rabbit and some other animals.

Some of the many muscles that may take part in mammalian breathing movements are so placed anatomically that a decision as to whether their contraction would enlarge the chest or decrease its size is difficult. If it enlarges the chest, it would be an inspiratory muscle, otherwise an expiratory one. A method suggested by Newell Martin has helped to determine the true function of certain respiratory muscles. In this method, contractions of the diaphragm of an anaesthetized animal are graphically recorded. Simultaneously, the respiratory contractions are also recorded for the muscle of which the function is to be determined. If the muscle contracts synchronously with the diaphragm, it is regarded as inspiratory; if it contracts alternately with the diaphragm, it is expiratory. The diaphragm is the chief muscle for mammalian respiration, although others are concerned to a varying degree.

The variations in the extent of lung ventilation are considerable. In man, for example, the air inspired or expired during a normal, quiet breathing movement is about 500 cc., as measured by the spirometer. This is the average for adults. It is called the **tidal air**. But the maximal air exchange, that is, the volume expired by a maximal compression of the chest after a maximal inspiratory effort, may be over 4,000 cc. The average for normal adult men is about 3,500 cc. This maximal output is called the **vital capacity**. It is sometimes used to nearly its full extent during the deep labored breathing that follows violent exercise.

The Alveolar Air.—The lungs are not completely emptied by any breathing movements. Even with extreme compression of the thorax the lungs are still large enough to hold about 1,000 cc. of air in the adult man. This has been shown by measurements of the air in the lungs of cadavers after the chest was forcibly compressed, the trachea tied and the lungs removed. The air remaining after forcible expiration is called the **residual air**. The air remaining in the lungs and respiratory passages after a quiet, unforced expiration, is much more than the residual air. There is thus a considerable amount of what is called the "dead space" which is the space in which the air is not actually renewed by a breathing movement. On this account the exchange of gases between the lungs and the atmosphere must be largely by means of diffusion rather than by actual air currents. Particularly is this true of the exchange between the atmosphere and the gases in the small chambers or **alveoli** of the lungs. These are the terminal sacs at the ends of the smallest of the air passages and are the parts of the lungs that furnish the best opportunity for exchange of gases between their contents and the pulmonary blood. The content of these small chambers is spoken of as the **alveolar air**. Haldane and his co-workers devised a method for obtaining expired air samples that represent very closely, if not exactly, the composition of alveolar air. In brief, this method consists in first expiring to nearly the greatest possible extent and then at the end of this maximal expiration taking

the sample of air. The alveolar air does not change perceptibly as the result of any one breathing movement, but retains a fairly uniform composition throughout any period of uniformity of muscular activity and of rate and depth of breathing. Inasmuch as the gaseous exchange of external respiration is chiefly between blood and alveolar air, a knowledge of the composition of the latter has been very helpful in understanding the physiology of respiration.

The Process of Gaseous Exchange in the Lungs.—The structure of the alveoli (Fig. 161) indicates the favorable conditions for gas diffusion between the pulmonary blood and the alveolar air. The wall of each alveolus is very thin and bears a close network of fine capillaries which are also very thin-walled. The capillaries are so small that the blood flows through each of them in a very fine stream, so fine indeed that the corpuscles must pass through in single file in most cases. It thus results that, although a drop of blood passes through the lung capillaries in a little less than two seconds, it is efficiently aerated.

Analyses of blood and alveolar air show that the nature of the exchange process is that of gas diffusion, at least under all ordinary circumstances. In order to make clear the nature of this process, the conditions which determine the diffusion of gases between gaseous and liquid phases must be reviewed.

A gas diffuses from any place where its **partial pressure** is comparatively high to one where it is lower. The partial pressure of any gas in gaseous mixtures is proportional to its concentration in the mixture. For example, oxygen composing approximately 21 per cent of air at a barometric pressure of 760 mm. Hg. exerts a partial pressure of about 160 mm. Hg. (0.21×760). This is often called the **oxygen tension** of air. The solubility of a given gas in water is proportional, at constant temperature, to the partial pressure or tension of the gas in the atmosphere to which the water is exposed. For example, pure oxygen at 760 mm. pressure dissolves in water to nearly five times the extent that it does when air is exposed to water at the same temperature and pressure. The tension of oxygen in air is only about one-fifth of what it is in an atmosphere of pure oxygen. A gas diffuses into a liquid which dissolves it and also diffuses out of the liquid into the gaseous phase. Gas molecules go into solution at a rate proportional to the concentration of the gas in the gaseous phase. Molecules of the gas pass out into the gaseous

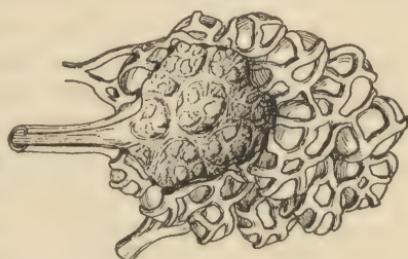


FIG. 161.—Diagrammatic representation of a single alveolus of the lung, suggests the net-work of capillaries surrounding a thin-walled sac. (From Stiles, "Human Physiology," W. B. Saunders Co.).

phase at a rate proportional to the concentration of the dissolved gas in the liquid phase. Equilibrium is reached, at constant temperature, when these two diffusion rates become so adjusted to each other that the concentration of the gas does not change in either phase. This equilibrium is really a dynamic and not a static one for the interchange of gas molecules is still going on. Any change in the partial pressure of the gas or in the concentration of its dissolved molecules alters the rate of the diffusion in one or the other direction so that a new equilibrium is reached.

In the case of blood gases, conditions are somewhat complicated in that O₂ and CO₂ are not merely held in solution but are also in certain kinds of chemical combination. These combinations very readily break down to give off their gases. The amounts thus combined are much larger, as will be shown below, than the amounts in solution. The concentration of gases in mere solution are therefore of little significance in determining the rates of their diffusion into and out of the blood. But the actual blood gas tension, that is, the comparative force with which the blood gases tend to diffuse out of the blood, can be measured. *The tension of any gas in solution is measured by finding the partial pressure of that same gas which may be present in the adjacent atmosphere without causing either increase or decrease in the amount of gas dissolved.* Such measurements have been carried out upon blood. The original method was developed chiefly by Krogh. The principle involved is simply that of finding a gaseous mixture with which the blood remains in equilibrium without change in its gas contents. A small sample of the blood is acidified to liberate all of its CO₂ and is then exposed to a vacuum. This removes all of the O₂ and CO₂ which can then be determined by suitable methods of gas analysis. A further set of small blood samples is then exposed, in air-tight chambers, to atmospheres containing known proportions of O₂ and CO₂, varied in the case of each sample. After each has come to equilibrium at constant temperature, the blood samples are analyzed for their gas contents. The particular one which has the same O₂ and CO₂ content as the original untreated blood sample must have been exposed to an atmosphere having the same tensions of these gases that prevail in the blood itself. In such measurements, the blood must be treated to prevent clotting (defibrinated or oxalated) so that results obtained by this method have been questioned as perhaps not representing the physiological condition of the blood. Another method, however, gives substantial confirmation. In this method one lung is blocked in the bronchial tube leading into it. In due time, the blood flowing through this lung comes into equilibrium with the stagnant air contained in it. Some of this air is then drawn off and analyzed. The partial pressures of its O₂ and CO₂ are thus determined and give a measure of the corresponding gas tensions in the blood of the occluded lung. This blood must of course be venous since air was excluded from it. Special methods

have been devised by Douglas and Haldane for determination of the oxygen tension of blood. These methods depend in principle upon the fact that a given sample of blood will take up carbon monoxide (CO) in proportions that vary in a known way according to the oxygen tension of the blood. By these and some other methods it has been shown that, under all ordinary circumstances, the tension of oxygen is as great or greater in the alveolar air than it is in the blood, even as it leaves the lungs in a highly arterialized condition. Also the tension of CO₂ in the alveolar air is less than that of the venous blood. Some representative figures are given in the following table.

GAS TENSIONS—EXPRESSED IN MM. OF HG

	In arterial blood	In alveolar air	In venous blood
Oxygen.....	100	100.00	37.6
Carbon dioxide.....	35	35.40	42.6

These figures show that oxygen can diffuse from alveolar air into venous blood until it becomes arterial and that carbon dioxide can diffuse from venous blood into the alveolar air. Such results have lead to the conclusion that the exchange of gases in the lungs is in accordance with the laws of simple physical diffusion. At any rate, conditions are favorable for gas exchange by mere diffusion. If this is the only process concerned, then the membranes of the capillary walls and the alveoli behave as inert structures that passively permit gas diffusion.

The Possibility of Oxygen Secretion by Lung Tissues.—Haldane has been the chief advocate of a different theory of the nature of gas exchange. He and other investigators obtained evidence to show that mere diffusion of gases cannot always account for the amounts of oxygen taken by the blood when the air has certain low oxygen tensions. This condition occurs in rarefied atmospheres, at high altitudes or in partially exhausted vacuum chambers. It produces the condition of partial oxygen starvation, which is called **anoxaemia**. The symptoms of slight oxygen deprivation are usually called mountain sickness and include dizziness, headache, unusual susceptibility to fatigue, a tendency to nausea and sometimes fainting. Under these circumstances, the arterial blood is not provided with its normal content of oxygen. After a sufficiently long sojourn at high altitudes, a person may become acclimatized so that he no longer feels any difficulties, even during the performance of heavy muscular work. The process of acclimatization includes numerous physiological readjustments, such as increased numbers of red corpuscles, increased rate of circulation, and increased lung ventilation. But none of these

adjustments could account for a fact which has been repeatedly found to be true of such acclimatized persons, namely, the oxygen tension of their arterial blood is higher than that of the alveolar air. The laws of gas diffusion fail to apply to this condition. This suggests, as Haldane and others maintain, that the cells of the alveolar tissue actually secrete oxygen into the blood. This process is known to occur elsewhere in nature. Glands of the swim bladders of many species of fishes secrete oxygen under high pressure into the swim bladders. Other somewhat similar examples of oxygen secretion are found among certain invertebrates. The thin endothelial cells of the alveolar walls do not seem to be adapted, however, to carry on secretion and, as Bayliss and others have pointed out, explanations other than that using the idea of secretion are hypothetically possible. The problem awaits further investigation.

Changes in the Air and Blood during Respiration.—Analyses of inspired and expired air show that, during ordinary rates of breathing, the human body reduces the oxygen of the air by a little less than 5 per cent and raises the carbon dioxide by about 4.3 per cent. These figures vary with species and in the same individual under different circumstances. The following averages, compiled by Howell, are representative. Figures are in per cent by volume. Nitrogen of the air behaves as an inert gas

	N	O	CO ₂
Inspired air.....	79	20.96	0.04
Expired air.....	79	16.02	4.38
		4.94	4.34

toward the animal body. The same is true of the rare gases such as argon, helium, neon and krypton which are not determined in physiological analyses but are merely measured with the nitrogen. Carbon dioxide is added to the air in the lungs in an amount less than the oxygen withdrawn. This is due to the fact that some of the oxygen utilized for physiological oxidations constantly goes to form water, a part of which is excreted by the breathing. Moisture is always added to respiration air. It is evaporated to a slight extent from the lung tissues but most of it is evaporated into respiration air from the linings of the nasal passages, the mouth, pharynx and trachea. Even when the atmosphere is saturated with moisture, water vapor can still be added to it ordinarily, during respiration in warm blooded animals, because the temperature of the air is usually raised as it passes into the respiratory system. The condition is different, of course, when the air is at body temperature and saturated with vapor. But this, as most persons know from experience, creates

considerable discomfort. This is one reason why cool and moderately dry air is favorable to comfort and physiological well being.

Blood passing through the lungs changes from the venous to the arterial condition. The explanation of the accompanying change in blood color was given in the preceding chapter. The essential facts are shown by the result of analyses of the gases which may be pumped off into a vacuum from acidified samples of arterial and venous blood. These results vary considerably according to the species of animal and the condition of the individual. The results of such analyses are usually expressed as cubic centimeters of the various gases obtained from 100 cc. of the blood. Average figures for human blood are the following:

	O	CO ₂	N
Arterial blood.....	20	38	1.7
Venous blood.....	12	45	1.7
Difference.....	8	7	0

Variations are determined chiefly by: (1) The relative amount of muscular activity, (2) the relative amount of lung ventilation, that is, the rate and depth of the breathing movements and (3) the character of the food substances that are undergoing oxidation in the body. All these variations are surprisingly small in the blood of any one individual although the variations may be considerable between different individuals even of the same species. It appears, however, that in any given individual the composition of the blood in any particular portion of the circulation maintains remarkable constancy. This is possible because of the nervously regulated adjustments of the heart beat, of the blood pressure, of the blood supply to each part of each organ and of the rate and depth of the breathing movements. Regulation of the circulation was described in the preceding chapter. Regulation of the breathing will be discussed below. The gases of venous blood are present regularly in varying amounts in different veins of the same animal. For example blood of the femoral vein, which conveys blood from the leg muscles, has less oxygen and more carbon dioxide in it than in that of venous blood coming from the brain. These differences are due to the relative rates of oxidation in different tissues and to the relative rapidity of blood flow or the amounts of blood provided to the tissues. The rate and amount of blood flow to any given tissue are determined by vasmotor adjustments. Rate of oxidation is partly determined by inherent prop-

erties of each kind of tissue and partly by the amount of excitation to which it is responding momentarily. The blood entering the lungs is a mixture of venous bloods from the entire systemic circulation. It therefore tends to have a comparative constancy of gaseous content. This tendency approaches to a complete constancy of regulation because of the nervously controlled adjustment of circulation and respiration.

The Condition of Gases in the Blood.—Nitrogen appears to be merely dissolved in blood plasma. This explains why the proportion of atmospheric nitrogen taken by the blood is very small. Oxygen is held chiefly in combination with hemoglobin. This is clearly shown by comparison between the amounts of oxygen that can be taken up by blood plasma and by whole blood. In 100 cc. of plasma only about 0.3 cc. of oxygen can be held. In 100 cc. of whole blood more than 20 cc. of oxygen can be held. Carbon dioxide is held chiefly as bicarbonates. As these are electrolytically dissociated, the HCO_3^- ions are the chief form of this gas in the blood. Sodium bicarbonate dissociates thus,



The relative abundance of sodium in the blood indicates that most of the bicarbonate is present as the sodium salt. Potassium and other metallic bases may compose a minor part of the bicarbonate. Theoretically, some CO_2 of the blood might be in the form of merely dissolved molecules and of carbonic acid molecules. This must be a very small part, however, because the slight alkalinity of the blood represents a considerable excess of weak bases over acids and these bases, in accordance with the law of mass action, convert nearly all of the CO_2 into bicarbonates. This is further confirmed by the smallness of the part of the blood CO_2 which may be removed by the vacuum pump without adding acid to break down the bicarbonates of the blood.

Hemoglobin as an Oxygen Carrier.—Hemoglobin reacts with oxygen in a way that is remarkable in comparison with other chemical reactions. Hemocyanin, the blue copper-containing protein of the blood of certain crabs, is also a respiratory pigment in that it, like hemoglobin, is an oxygen carrier. But even hemocyanin, according to the work of Alsberg and Clark, does not show the peculiarities that characterize hemoglobin. The reaction, in which hemoglobin unites with oxygen to form oxyhemoglobin, behaves in some ways like a true chemical reaction and in others like a physical process. Its similarity to chemical reaction is found in the observation that when hemoglobin is thoroughly exposed to oxygen-rich air it is completely converted into oxyhemoglobin so that further exposure to pure oxygen under high pressure does not further increase the amount of oxygen in combination with hemoglobin. In other words, hemoglobin reaches what is called its saturation point. Moreover,

the amount of oxygen taken up by hemoglobin at its saturation point is just equal to that which represents two atoms of oxygen for every one atom of iron in hemoglobin. On the assumption that hemoglobin contains one atom of iron in its molecule, one molecule of O_2 combines with one of hemoglobin. This is analogous to the formation of iron peroxide, FeO_2 . These combining proportions of oxygen and hemoglobin are further established by the fact that when the molecular weight of hemoglobin is estimated from the amount of oxygen that it takes up at saturation, the resulting figure comes out very close to 16,669. This, as explained above, is the most probable figure for the molecular weight of hemoglobin. These facts do not necessarily prove that oxygen merely unites with the iron atom when oxyhemoglobin is formed. In fact, the case is probably much more complex than this. For one thing,

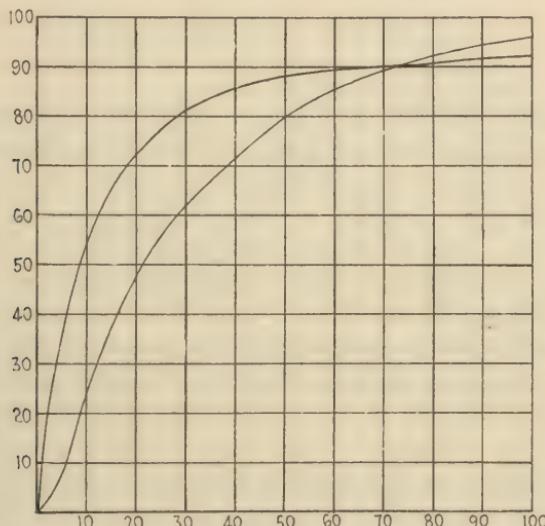
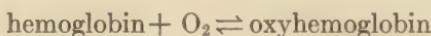


FIG. 162.—Dissociation curves of hemoglobin. Abscissas are tensions of O_2 in the surrounding air, expressed in mm. Hg.; ordinates are percentages of saturation of hemoglobin with oxygen. The upper curve is for hemoglobin in the absence of salts, that is after its solution has been dialyzed; the lower one is in the presence of blood salts. (After Barcroft and Roberts).

hemoglobin and oxyhemoglobin are very differently affected by processes of acid hydrolysis. When hemoglobin is broken down by acids, **hematoporphyrin**, an iron-free compound, is liberated. But the corresponding substance, similarly obtained from oxyhemoglobin is **hematin**, which contains all the iron of the original oxyhemoglobin. This hematin does not behave, however, like oxyhemoglobin, for it does not give off its oxygen to a vacuum, although yielding it readily to reducing agents. This shows that the oxygen-combining power of the iron in hemoglobin is in some way modified by parts of the molecule other than the iron-con-

taining radical. Other observations, which need not be detailed here, also lead to uncertainty as to just how oxygen combines with hemoglobin. Yet the chemical nature of this union is clearly indicated. On the other hand, one fact clearly distinguishes this reaction from ordinary ones. The proportion of oxyhemoglobin to hemoglobin, present in the blood at any time, is dependent upon the tension of O_2 in the blood. This is shown by the so-called dissociation curve of oxyhemoglobin. (Fig. 162.) In this curve, the percentage saturation of hemoglobin with oxygen is taken as ordinates and the oxygen tension as abscissas. The curve shows that at any given oxygen tension the process of oxyhemoglobin production comes to an equilibrium just as truly as the rise of water in vertical pipes comes to an equilibrium that is determined by the "head" or pressure of the water supply. In view of its ready reversibility, the reaction:



bears a resemblance to a physical process.

Temperature affects the dissociation curve of oxyhemoglobin. The higher the temperature, the less is the amount of oxygen combined with hemoglobin at any given oxygen tension. In other words, rise of temperature throws the reaction, as represented above, in the direction from right to left. This effect resembles that of temperature upon electrolytic dissociation, but it should be understood that the dissociation of oxyhemoglobin is not an electrolytic one. This temperature effect is in marked contrast to those exerted upon true oxidation reactions. In the latter, rise of temperature hastens the union of oxygen with the oxidizable substances. Dissociation of oxyhemoglobin with rising temperature does resemble one process, however that is, the dissociation of certain so-called adsorption compounds, such as starch iodide, which is broken down to starch and free iodine by a rise in temperature. Future investigations may show that oxyhemoglobin and the adsorption compounds are of the same nature.

The presence of electrolytes also affects the dissociation curve of oxyhemoglobin. The curve obtained with pure crystallized oxyhemoglobin is distinctly different from that obtained with blood. The curve obtained with blood which has been dialyzed to remove its salts is also different from that obtained with blood itself (Fig. 162). At the lower oxygen tensions, oxyhemoglobin dissociates more completely in the presence of salts than it does in their absence. This has significance for the oxygenation of body tissues. As the blood passes through systemic capillaries where oxygen tension is very low, the electrolytes of the blood favor the release of oxygen for tissue utilization.

Acids also affect the dissociation curve. Increase in hydrogen ion concentration favors the dissociation of oxyhemoglobin. This effect

has been studied chiefly in relation to CO_2 , the most abundant acid substance in the body. Figure 163 shows the results of such studies. It indicates that increase of CO_2 tension markedly favors the liberation of oxygen from oxyhemoglobin. The significance of this in the process of respiration is apparent. Carbon dioxide entering the blood of the systemic capillaries favors the release of oxygen for utilization by the tissues, as will be shown in a later paragraph. This relationship is in a sense reciprocal, that is, the taking on of oxygen by the blood, facilitates the giving off of CO_2 (p. 573).

The effectiveness of hemoglobin as an oxygen carrier is shown by the extent to which it combines with oxygen in the lungs. If the amount of

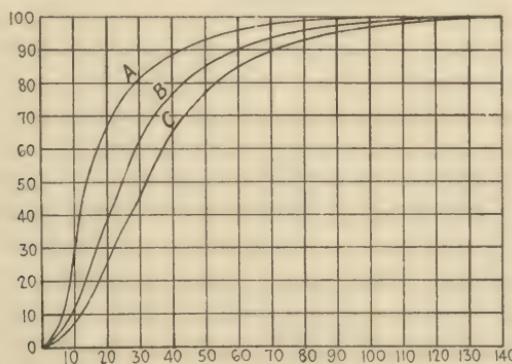


FIG. 163.—Effect of CO_2 on the dissociation curve of hemoglobin. Abscissas are O₂ tensions; ordinates, percentage saturation of hemoglobin with oxygen. A, curve obtained in the presence of CO_2 at 5 mm. Hg. tension. B, at 20 mm. Hg. tension. C, at 40 mm. Hg. tension. (After Bohr).

O₂ taken up by blood at equilibrium with pure O₂ is taken as complete oxygen saturation of the blood, then the amounts taken by normal human blood when arterialized in the lungs, is 94 to 96 per cent of saturation. This is about 2 per cent less than the maximum oxygen of blood that has come to complete equilibrium with normal alveolar air under atmospheric pressure. The variation between 94 and 96 per cent is chiefly determined by differences in individuals. The percentage oxygen saturation in arterial blood is very nearly uniform for the same person. These individual differences are attributed by Barcroft to differences in the hemoglobin.

The Respiratory Exchange in the Tissues.—As the arterial blood enters systemic capillaries it is nearly saturated with oxygen which is held under a tension not far from 100 mm. Hg. Carbon dioxide is present to an extent represented by a tension of about 35 mm. Hg. Though these figures are based chiefly upon analyses of human bloods, they are fairly representative of conditions in all mammals. Through

the walls of tissue capillaries, gases diffuse in the same way that they do through lung capillary walls. Diffusion must also occur across the lymph which is present to a varying extent around all capillaries. The actual gaseous exchange occurs between the blood and tissue cells. The tensions of O_2 and CO_2 in the cells are not exactly known. They are no doubt widely variable. In view of the above-mentioned fact, that cells require constant renewals of oxygen supply thus giving evidence that they possess no oxygen store, it seems likely that oxygen tension in the tissues is not far from O. The CO_2 tension of the tissues varies with their relative activity and its accompanying oxidation and is therefore different in different tissues. But in any case, CO_2 tension is never very low in living tissues especially in those of warm blooded animals in which CO_2 tension is estimated to vary between 50 and 70 mm. Hg. in various tissues. Conditions, then, are such as to cause the diffusion of O_2 from blood to tissues and of CO_2 from tissues to blood.

Even the venous blood, emerging from active tissues, is far from being oxygen-free. Its hemoglobin is still partly in the form of oxyhemoglobin. Venous blood contains O_2 in amounts equivalent to about 60 per cent of saturation. From 100 cc. of venous blood about 12 cc. of O_2 can be removed by a vacuum pump. This failure of the tissues to remove all of the oxygen in their blood supply is not surprising if one remembers that the excess of oxygen supply over demand secures oxygen for the cells at a certain high tension. This probably is a distinct advantage for physiological processes.

Changes in carbon dioxide content of the blood are also within confined limits. The arterial blood contains some 35 volumes per cent of CO_2 even when lung ventilation is very good.

The Oxygen Consumption of Tissues.—Various methods for measuring the rate at which oxygen is used by the tissues of specific organs have been devised. In principle, they all consist in taking samples of blood from large arteries while the venous blood flowing away from the organ under investigation, is simultaneously sampled. The two bloods are analyzed for their content of gases. Results are computed in terms of oxygen used per gram of the organ per minute. Such computations require information concerning the rate of blood flow through the organ at the time the samples are taken. When this rate and also the rate of oxidation in the organ are fairly constant the computations are significant.

These figures are compiled from the work of numerous different investigators. Although the absolute values may not be perfectly representative, the comparative values are significant. They show, for one thing, that the rate of oxidation is distinctly higher in some tissues than in others. The comparatively high oxygen utilization of the adrenal glands, for example, is noticeable. For another thing, these results give a pic-

ture of the increase in rate of oxidation that accompanies increased activity. This latter relationship has been studied more particularly in the case of the heart. It has been shown, chiefly by the work of Rohde, that the oxygen used per minute is proportional to the product of the heart rate and the maximum tension produced by the heart muscle. This is equivalent to saying that the heart uses oxygen in proportion to the work which it does.

OXYGEN USED BY VARIOUS ORGANS

Figures are cc. of O₂ used per gram of tissue per minute

	In a resting or comparatively inactive condition	In a condition of excitation to activity
Adrenals.....	0.045	0.150 (during high blood pressure)
Heart.....	0.043	0.085
Submaxillary gland.....	0.027	0.089
Kidney.....	0.026–0.060	much increased during increased rate of urine secretion.
Lung.....	0.015	
Liver.....	0.005–0.018	0.025–0.050
Intestines and other viscera.....	0.008–0.013	0.011–0.018

The oxygen consumption of nervous tissue has not been determined very satisfactorily. The results usually obtained are lower than those for muscle yet the quick loss of consciousness which follows deprivation of oxygen shows that a plentiful oxygen supply is required for intense activity of the brain. Possibly, nervous tissue, at least for its conscious activities, requires that oxygen be supplied at high tension even though the actual oxygen utilization may be comparatively small. The very small oxygen utilization in nerve trunks was discussed above (p. 275).

The Transport of Carbon Dioxide by the Blood.—It is remarkable that blood can take up large quantities of CO₂ and yet maintain its reaction with such constancy that its pH varies only by a barely detectable amount. Human blood has slightly different hydrogen ion concentrations in different individuals. Results have been reported as high as pH = 7.25 and as low as pH = 7.40. But for any given individual, the hydrogen ion concentration of the blood is remarkably constant and is not much changed by the additions of CO₂ that occur in the tissues. For example, an arterial blood of which the pH = 7.35 (the average for human beings) may change as it becomes venous to pH = 7.34 or possibly to pH = 7.32. But if an amount of CO₂ comparable to that in venous

blood were dissolved in pure water, the pH of the latter would change from 7 to approximately 4.5. The mechanism of the buffer action which prevents any great change in the hydrogen ion concentration of blood was discussed in Chap. VII. This mechanism was shown to include the bicarbonate system, the phosphate system and the blood proteins. That discussion applied more particularly to the blood plasma and similar body fluids, such as the lymph, than it did to whole blood. In the latter, the regulation of hydrogen ion concentration depends not only upon substances dissolved in the plasma but also upon those held within the blood corpuscles. The manner in which these latter substances influence the CO_2 transport by venous blood largely accounts for the fact that its hydrogen ion concentration is little or no greater than that of arterial blood.

As CO_2 tends to accumulate in the blood plasma of the systemic capillaries, it increases the concentration of NaHCO_3 in the plasma. The Na ions required for the formation of this bicarbonate appear to be furnished by the NaCl of the blood. At any rate, no other source could supply as adequate amount of these ions. This fact has been appreciated only recently. Its truth was not surmised earlier because it would seem that chlorine ions would be left free and thus produce acidity in the blood. But from the work of numerous investigators, it is now known that when the blood changes from arterial to venous there is a shifting of chlorine ions from the plasma to the red corpuscles. This fact has been proved by exposing defibrinated blood to various tensions of CO_2 , separating serum from corpuscles and then determining chlorine in the ash of both serum and corpuscles. These analyses show that the higher the CO_2 content of the blood, within physiological limits, the more is the chlorine transferred from the liquid part of the blood to the corpuscles. It must enter the corpuscles in the form of HCl (or, more strictly speaking, as H^- and Cl^- ions). This appears to be the case because if H_2CO_3 reacts with NaCl , HCl is formed. This reaction does not occur to any significant extent, when CO_2 is dissolved in NaCl solution; but it does occur to a significant extent in the blood, because the shifting of HCl into the corpuscles as fast as it is produced in the plasma, facilitates the reaction.

This raises the question as to what becomes of the HCl after it enters the corpuscles. An answer to this question has been found in a study of the behavior of hemoglobin and oxyhemoglobin. They occur in the corpuscles in the form of some combination with metallic bases. As potassium is by far the most abundant base in the corpuscles, hemoglobin and oxyhemoglobin may be regarded, for purposes of description, as potassium-containing compounds. But these, like all such protein compounds react with acids. It thus results that the HCl which enters the corpuscles is largely neutralized. Hemoglobin and oxyhemoglobin

react with HCl to form neutral KCl and potassium-free hemoglobin and oxyhemoglobin. The free proteins combine with most of the hydrogen ions liberated from HCl because these proteins are weak acids which do not greatly dissociate. They thus act as buffers within the corpuscles. This buffer action is especially facilitated by the change of oxyhemoglobin to hemoglobin during the passage of blood through the systemic capillaries. This facilitation results from the very weak acid dissociating power of hemoglobin as compared with that of oxyhemoglobin. In other words, hemoglobin is a much weaker acid than is oxyhemoglobin. This fact has been established by determinations of the hydrogen ion concentrations of arterial and venous blood under a given CO_2 tension. Such determinations show that venous blood is a better buffer for a given amount of carbonic acid than is arterial blood. The reduction of oxyhemoglobin is shown by Van Slyke's computations to be very significant. This change might even make it theoretically possible for blood to take up the amounts of CO_2 which enter it in the systemic capillaries without any change of its pH. Van Slyke estimates that hemoglobin, as it is reduced in the corpuscles, actually accounts for the neutralization of no less than 80 per cent and possibly for as much as 95 per cent of all the CO_2 added to the blood as it changes from arterial to venous. Thus the giving off of O_2 to the tissues, that is, the reduction of oxyhemoglobin, facilitates the taking on of CO_2 by the blood. The peculiar properties of hemoglobin are no less useful in the process of CO_2 transport than they are in O_2 transport.

The phosphates of the corpuscles also exert some, though a subordinate, buffer action. Its amount can be estimated from the proportion of potassium phosphate present in the corpuscles. It is probably more effective as a buffer within the corpuscles than it is in the plasma; but in any case, its significance as a CO_2 carrier is not great.

Although most of the CO_2 taken into the blood from the tissues remains in the plasma as bicarbonates, a part of it shifts into the corpuscles. This is shown by the fact that less CO_2 is obtainable from the separated plasma of venous blood than from separated plasma of arterial blood after it has been exposed to a CO_2 tension equivalent to that of venous blood. By measurements of this difference, it has been shown that one-fourth to one-third of the CO_2 , added to the blood in the systemic capillaries, enters the corpuscles. It goes in as H_2CO_3 or as H^+ and HCO_3^- ions. Cells are even more permeable to carbonic acid than they are to hydrochloric acid. Upon entering the corpuscles, H_2CO_3 reacts with hemoglobin, oxyhemoglobin and phosphates in the same way that HCl reacts.

A summary of some of the reactions by which the corpuscles are enabled to aid in CO_2 transport and simultaneously to check the acidifying effect of CO_2 , is given by Van Slyke in the following schematic form.

Plasma	Cell wall	Cell
(1) $\text{H}_2\text{CO}_3 + \text{NaCl} \rightleftharpoons \text{NaHCO}_3 + \text{HCl}$	$\rightarrow \text{HCl} \rightarrow$	(3) $\text{HCl} + \text{K}_2\text{HPO}_4 \rightleftharpoons \text{KH}_2\text{PO}_4 + \text{KCl}$ (4) $\text{HCl} + \text{KHbO} \rightleftharpoons \text{HHbO} + \text{KCl}$ (5) $\text{HCl} + \text{KHb} \rightleftharpoons \text{HHb} + \text{KCl}$
(2) $\text{H}_2\text{CO}_3 + \text{NaProtein} \rightleftharpoons \text{NaHCO}_3 + \text{HProtein}$	$\rightarrow \text{H}_2\text{CO}_3 \rightarrow$	(6) $\text{H}_2\text{CO}_3 + \text{K}_2\text{HPO}_4 \rightleftharpoons \text{KH}_2\text{PO}_4 + \text{KHCO}_3$ (9) $\text{H}_2\text{CO}_3 + \text{KHbO} \rightleftharpoons \text{HHbO} + \text{KHCO}_3$ (8) $\text{H}_2\text{CO}_3 + \text{KHb} \rightleftharpoons \text{HHb} + \text{KHCO}_3$

In these reactions, "Na Protein" refers to all the plasma proteins in alkali combination. It does not take into separate account the minor contingent of plasma proteins combined with other bases such as K and NH₄. "H Protein" refers to free plasma proteins not combined with bases. "KHb" and "HHb" are used similarly for the alkali combinations of hemoglobin and free hemoglobin. "KHbO" and "HHbO" similarly refer to oxyhemoglobin in its two forms. Combinations of hemoglobin and oxyhemoglobin with bases other than potassium are disregarded.

Reaction (1) accounts for the way in which the major part of the CO₂ is actually carried in the blood. Reaction (2), together with some other subsidiary ones, represents the disposal of a small part of the CO₂. The shift of H₂CO₃ into the corpuscles is a significant factor in the distribution of the CO₂ load which the blood must carry. Reactions (5) and (8) probably represent the most important process in the stabilizing of the C_H of blood as it becomes venous.

In the lungs, a reversal of these various reactions occurs. As CO₂ is given off by the blood to the alveolar air, the various bases, previously liberated from buffers to form bicarbonates in venous blood, are restored to the buffers. In this process, the oxidation of hemoglobin plays a conspicuous rôle. Inasmuch as oxyhemoglobin is a stronger acid than hemoglobin, oxygen intake by the corpuscles tends to drive H₂CO₃ and HCl out of the corpuscles. HCl, thus entering the plasma, facilitates the liberation of CO₂ from its bicarbonates. Phosphates and plasma proteins also take some part in the liberation of CO₂. Bicarbonates would not give off to alveolar air the amounts of CO₂ that are regularly expired unless they were assisted by the other buffer substances of the blood. This has been proved in part by studies of the dissociation of pure bicarbonate solutions and in part by studies of the CO₂ evolved from corpuscle-free blood serum under known CO₂ tensions. The blood corpuscles are

thus shown to be just as important for the giving off of CO₂ in the lungs as they are for the taking on of O₂.

The Regulation of Respiration.—The muscular movements of breathing need to be regulated so that lung ventilation shall vary with the composition of the blood. Without this coordinated regulation the O₂ supply of the body could not be increased when tissue activity increased nor could removal of CO₂ be speeded up whenever it tended to accumulate in the blood. The coordination between respiration and blood composition is effected by the respiratory center (p. 378). As shown by Haldane and Priestley (1905) it is the CO₂ tension of the arterial blood which determines the rate at which the respiratory center discharges the nervous impulses which control the muscles of respiration. The center really controls both the rate and the depth of the breathing movements and thus determines the lung ventilation. The latter determines the composition of the alveolar air. The CO₂ tension of the latter determines the CO₂ tension of the arterial blood. Breathing regulation is therefore a compensatory type of adjustment. Increase of CO₂ in the blood increases the rate and depth of breathing, this increases the lung ventilation so as to reduce the CO₂ tension of the blood and this slows down the breathing.

The Ineffectiveness of Lowered Oxygen Supply.—It might seem at first thought that since respiration is the means for securing an oxygen supply to the cells, the relative O₂ tension of the blood would be the determining factor for the regulation of breathing. A simple experiment is sufficient to prove, however, that a marked poverty of oxygen in the blood exerts no effect upon respiratory movements, provided that the CO₂ tension of the blood is not altered. If a man breathes into an air-tight collapsible bag so arranged that the air exchanged between the bag and his lungs must pass through flasks containing sodium hydroxide, the outgoing carbon dioxide is absorbed by the alkali but no renewal of oxygen is possible. Under these circumstances the alveolar air and therefore the blood attain a low O₂ tension but retain approximately their normal CO₂ tensions. No effect on the breathing movements is produced. It is even possible to continue this experiment until the subject becomes blue in the face (cyanosis) from unwanted oxygen deprivation and consequent reduction of hemoglobin; but even then the rate of respiration is not altered. Unconsciousness and in some animals perhaps even death may result from oxygen deprivation without any speeding up of respiration as a result of the lack of oxygen. It is probable that oxygen deprivation does stimulate the respiratory center when the deprivation is extreme and prolonged. Possibly this effect is an indirect one resulting from a failure of oxidation in all the cells and even in the tissues of the respiratory center itself. This failure would result in the accumulation of acid products of incomplete metabolism. These would stimulate the respira-

tory center to increased activity. Such stimulation, however, would be very abnormal and would have no relationship to the normal stimulation of the respiratory center. Indeed, oxygen deprivation can be an excitant of the center only when pushed to disastrous lengths.

The Effects of Carbon Dioxide.—On the other hand, increase of carbon dioxide in the blood is a marked excitant of the respiratory center. This is shown by the simple experiment of breathing pure oxygen from a collapsible bag. In this experiment, with no carbon dioxide absorbers, the CO₂ tension in the alveolar air and the blood increases, while oxygen tension in the lungs and blood is prevented from decreasing by the supply of pure oxygen. The result is a marked increase in the rate of breathing. The effect of the increased CO₂ tension is exerted upon the respiratory center. This is shown by experiments such as the following. Two rabbits are anaesthetized and the blood from the carotid artery of each one is transfused into the carotid artery of the other. The blood of one of them is then made to have a high CO₂ tension either by clamping the trachea or by blowing carbon dioxide into the lungs. The result is that the other rabbit shows labored breathing because its brain is supplied with blood of high CO₂ tension. The rabbit with obstructed trachea shows normal breathing movements because its brain is supplied with blood of normal CO₂ tension.

Low CO₂ tension of the blood causes a decrease of the lung ventilation. A period of voluntary deep breathing, for example, so lowers the CO₂ tension of the alveolar air, and therefore of the blood, that the respiratory center temporarily stops sending impulses to the respiratory muscles. On this account, one can hold the breath easily during a surprisingly long period after such deep breathing. A similar experiment can be tried upon animals. An anaesthetized dog is given forced breathing by artificial respiration with a bellows or a pulmometer attached to an opening in the trachea (a tracheotomy). This can be continued long enough to markedly reduce the CO₂ tension of the blood. If artificial respiration is then stopped, breathing movements are not resumed until oxidation in the animal's tissues has restored the normal or nearly normal CO₂ tension of its blood. Artificial respiration can even be pushed so far that after it is stopped, the animal fails to breathe for so long a time that it dies from oxygen starvation. In other words an animal can be killed by the indirect effects of over prolonged deep breathing. The condition of lowered CO₂ tension in the blood is called **acapnia** (without smoke) when it is sufficiently marked to cause the cessation of breathing. Acapnia is said to lower the excitability of the respiratory center. The resulting stoppage of breathing is called **true apnoea**.

Hydrogen Ion Concentration the Actual Regulator.—The effect of carbon dioxide on the respiratory center is really the effect of hydrogen ion concentration of the blood. It is known that any acid introduced into

the blood which bathes this center exerts a marked stimulus upon it. The effects of lactic acid and of hydrochloric have been especially tested. The relation of the C_H of the blood to the respiratory center was further tested by Hasselbach. He studied the effects of diet upon the hydrogen ion concentration of the blood. A high protein diet which produces potentially acid substances in the body was fed to an animal so as to make it secrete an unusually acid urine. This condition of the urine indicated that the blood would show some increase in its C_H unless the body made some compensatory adjustment. This adjustment was shown to be a change in the ventilation of the lungs. The alveolar air of this animal had a lower CO_2 tension on the high protein diet than on a low protein diet. This indicates an increased lung ventilation during the high protein feeding. The pH of the blood varied on the two diets only within the limits of the experimental error of hydrogen electrode measurements. This experiment shows that the respiration is so adjusted as to preserve the reaction of the blood within very narrow physiological limits. In other words, the respiratory center responds to minute changes in the blood C_H and responds in such a manner as to regulate lung ventilation, and therefore carbon dioxide elimination, to just the extent required for preservation of the normal blood reaction. This indicates that the respiratory center is far more sensitive, in its response to minute changes in the blood C_H than are any of the methods used for laboratory measurement of the blood C_H . Haldane estimates that a decrease of 0.012 in the pH of the blood causes a 100 per cent increase in the ventilation of the lungs. Much smaller changes in lung ventilation have been observed to occur in a definite and apparently well regulated manner, so that the respiratory center may be sensitive to changes of pH less than 0.001.

Reflex and Voluntary Effects.—Although the CO_2 tension of the blood is the chief factor in the regulation of the respiration, reflex effects have at least modifying influences. The effect of cold water on the skin, causing at first a tendency to hold the breath and later stimulating rapid or deep breathing, is familiar. All temperature conditions, both outside and inside the body, influence the respiration. Outside conditions do this in part by reflex effects. Pain is known to be accompanied in most individuals by increase in the rate of respiration. Artificial stimulation of afferent nerves in general, and especially of those containing pain fibers from the skin region, exert marked effects on the respiration. The effect is usually deeper breathing. Many kinds of emotional states are accompanied by marked changes in the rate and depth of breathing. Moreover, an effort of the will can produce breathing movements that are abnormally deep or rapid or both. This voluntary increase in lung ventilation can be kept up even until unconsciousness intervenes, due probably to effects on the vasomotor center. On the other hand, one

cannot hold the breath voluntarily beyond a certain point. The **inner stimulus**, as it is called, finally affects the respiratory center so powerfully that impulses must break through voluntary inhibition and start up respiratory movements. This inner stimulus is dependent upon the C_H . of the blood and is prepotent over certain reflex excitations of the center. All of the latter are, in a sense, merely modifying effects, superimposed upon the inherent rhythmic excitation occurring in the center itself.

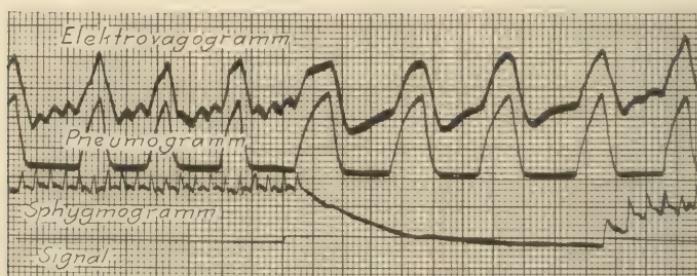


FIG. 164.—Tracings to show electrical variations in the vagus nerve of the dog as an accompaniment to respiration and the heart beat. The elektrovagogram shows electrical variations in the vagus nerve; the pneumogram records respiratory movements; the sphygmogram shows the heart beats. At the point indicated by the signal line, the heart was inhibited by excitation of the other vagus. The small waves on the elektrovagogram cease simultaneously, although the larger waves, concurrent with the uninterrupted respiration, continue. (After Einthoven, from Bayliss, "Principles of General Physiology," Longmans, Green & Co.).

One set of reflexes, however, acts rhythmically through the respiratory center and exerts a constant modifying effect upon respiration. These are reflexes aroused by *pressure conditions in the lungs*. The afferent fibers, concerned, are in the vagus. On this account, cutting of the vagi always alters, somewhat, the character of respiratory movements. The rate decreases and each breathing movement becomes deeper. The actual lung ventilation may not be significantly altered. But when the vagus nerves are intact, pressure conditions in the lungs noticeably affect breathing. When the lungs are partially deflated by strong suction applied to the trachea, a marked inspiratory movement is reflexly produced. It may even amount to a prolonged tetanus of inspiratory muscles. Correspondingly, when the lungs are strongly inflated, by a bellows attached to the trachea, a decided expiratory effort is produced reflexly. This latter effect appears to occur during every respiratory movement. This is indicated by Einthoven's records of the electrical variations of the vagus nerve. (Fig. 164.) Such a record is called a **vagogram**. It shows an electrical disturbance that corresponds to each respiratory movement. As the lungs expand during inspiration, pressure is exerted upon the lung receptors of afferent nerves. The resulting impulses reach the respiratory center by way of the vagus and tend to cut short

the inspiratory stimuli that are leaving the center. Thus there is maintained a sort of balance between the strength of the inner stimulus in the center and that of these particular reflex stimuli.

Reflex effects upon respiratory movements sometimes cause an increase of inspiratory effort without affecting expiration. Under other circumstance, expiratory muscles are reflexly excited without corresponding effects on the inspiratory ones. These observations indicate that the respiratory center is double. It probably consists of two parts, an inspiratory center and an expiratory center. Either of these may be subjected to reflex excitation or inhibition.

Excretion through the Lungs of Gases Other than Carbon Dioxide.— Nitrogen is sometimes given off in slight excess of that absorbed from the air. This may be due to changes in atmospheric pressure. Under high pressure the blood dissolves more nitrogen than it does under low pressure. If, therefore, an animal or a man is under a diminishing atmospheric pressure, nitrogen may be present in the expired air in amount slightly in excess of that inspired. But this excess does not represent any metabolic process and therefore has nothing to do with the true respiratory exchange. When this and other sources of experimental error are taken into account, nitrogen is found to have no significant, if any part in respiratory exchange. Excretion of nitrogen might occur theoretically through the lungs of herbivores. In their digestive organs, denitrifying bacteria are commonly found and are known to liberate nitrogen from the nitrates or nitrites that are present in the intestine. But the amount of these compounds in foods is relatively small. Most of the nitrogen, which is known to be present in intestinal gases, represents that of the air swallowed with the food. Experiments have not satisfactorily demonstrated that, even when nitrogen is produced by intestinal bacteria, it is actually excreted by way of the lungs rather than by way of the anus.

Other gases produced by intestinal bacteria have been shown to be excreted by way of the lungs. Hydrogen and marsh gas have been detected in expired air. In some cases, the major part of these intestinal gases is excreted through the lungs rather than through the anus. The total amount of these gases produced in the digestive system may be considerable in some animals. Boycott and Damant found as much as 375 cc. to be produced per hour as the average for a goat of 20 kilos. Along with these gases, large and variable quantities of CO₂ are produced by intestinal fermentations, amounting in some cases to 10 per cent of the total CO₂ production of the animal. The gas-producing fermentations in the intestine therefore cause serious difficulties in the estimation of the true oxidative exchange of herbivores.

Acetone, though a liquid at body temperature, is easily volatile. It is produced under the conditions of faulty oxidation prevailing in acidosis.

These conditions will be described in the next chapter. Acetone can be excreted by way of the lungs. Its odor is easily detected in the breath of persons suffering from acidosis in aggravated form.

Ammonia is found not infrequently in the air expired by human beings, but there is no conclusive evidence that it is excreted through the lungs. Indeed, the balance of the evidence is against this idea. Ammonia is probably formed by the action of bacteria in the respiratory passages. The case is different in certain invertebrates in which ammonia is an abundant product of metabolism. Weinland found ammonia and volatile amines to be regularly present in the expired air from the larvae of blowflies.

Foul odors of the breath are usually attributed to bacterial decomposition occurring in the teeth and the gums and upon the mucous surfaces of the tongue, pharynx and respiratory passages. To what extent, odors may be present in the breath as a result of the absorption of products of intestinal putrefaction is not known, but the breath may be free from any such odors even when intestinal putrefaction is known to be intense. In any case, volatile substances which are detected by the sense of smell are present in expired air in amounts too small to be detected chemically. The unpleasant odor of an overcrowded room is due more to volatile substances from sweaty, unwashed bodies than to expired air.

Toxins have been alleged to be present in expired air. Evidence of their supposed occurrence has been obtained by injecting water through which expired air had passed. The resulting symptoms of intoxication are such as would be produced by the injection of minute amounts of a foreign protein. Weichardt, who has been the chief investigator in this field, has shown, however, that the toxic substance is not really expired but is carried off in the form of minute droplets from the walls of the respiratory tract. The only real danger involved in breathing air expired by other individuals is due to these droplets. They may contain pathogenic bacteria. Such droplets are abundant and liable to be widely distributed during violent expiratory acts such as coughing or sneezing.

Ventilation.—On account of the discomfort produced in human beings under conditions of unsatisfactory ventilation, this subject has received a great deal of attention. Discomfort is not due to deficient O₂ supply nor to increased CO₂ tension in the air. Both of these conditions may be sufficiently extreme to be disastrous, but the oxygen of the air must be reduced to 14 per cent or less in order to produce any detectable effects upon human beings, while the CO₂ of the air must be raised to about 6 per cent before discomfort is experienced. Changes of this magnitude do not occur in cases of so-called poor ventilation. Even at an O₂ concentration of 16 per cent of the air, a person feels no discomfort and is even surprised to find that a match will not burn because of the low O₂.

content of the air he is breathing. The blood can still take up oxygen by virtue of the peculiar properties of hemoglobin and can take enough oxygen to supply the tissue needs. Also high CO₂ concentrations up to about 5 per cent of the air are without unpleasant effects. Breathing is faster and deeper but the resulting increase in lung ventilation prevents any disastrous or even uncomfortable effects from changes in blood composition. The actual cause of discomfort has been investigated chiefly by Leonard Hill and his co-workers. The temperature and the moisture content of the air are the important factors. In general, a high moisture content has a depressing effect and the more nearly the atmosphere is saturated with moisture, the more is the discomfort of abnormally high temperature felt. But even warm moist air causes no feeling of discomfort if it is kept in rapid motion. Currents of air passing over the skin facilitate evaporation and cause, possibly, other favorable effects. On this account, fanning can do away with feelings of discomfort even though the surrounding air is confined in an air-tight chamber and its composition is not altered during the increased movement.

The Effects of High Pressures.—The use of diving bells and caissons is increasingly common in modern engineering work. Caissons are steel chambers in which men can do construction work under water. Caissons are filled with air under sufficient pressure to keep water out of the excavations that are to be made or the masonry or other structures to be placed. Pressure must be twice that of the atmosphere at a depth of about 33 feet and must be 4 atmospheres at 100 feet. Such high pressures do not cause any marked discomfort provided the occupant of the caisson can equalize pressure on the two sides of the tympanic membrane of the ear. In all normal persons this is easily accomplished by swallowing. The compressed air is dissolved in the blood, however, in greater amounts than at atmospheric pressure. In the case of nitrogen, which composes about four-fifths of the air, the amounts dissolved in the blood under these conditions are very considerable. They are so great that, when the person is suddenly decompressed, the blood effervesces, as does an uncorked bottle of water that has been charged with gas under high pressure. Bubbles, which are composed chiefly of nitrogen, can be clearly seen in the tissues of an animal killed after it has been under high pressure and then suddenly decompressed. Such bubbles are very disastrous in their effects. The symptoms in human beings include dizziness, muscular pains, violent muscular cramps and oftentimes fatal apoplectic seizures. The after effects of non-fatal cases often include stiffness and soreness of muscles and joints. For this reason, these effects are popularly known among workmen as "the bends." The condition is also called **caisson disease**. The fatal effects are due to stoppage of the circulation at points where gas bubbles become lodged in the arterioles and capillaries of some part of the heart or the central nervous system. Necrotic

tissue has been found, especially in the nervous system, at autopsies upon victims of caisson disease.

A means for prevention of this difficulty and for relief of the condition when it occurs, has been found. It consists of a process of slow decompression. In a chamber under high pressure the dissolved blood gases remain in solution or if in bubble form are redissolved. If now the pressure in the chamber is slightly reduced, the blood gases come to equilibrium with the air at this lower pressure without forming bubbles in the circulation. By successive reductions in the pressure, with a period of waiting after each reduction, the occupants of the chamber are finally brought in safety to atmospheric pressure.

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CHAPTER XXI

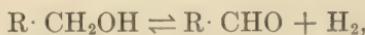
PHYSIOLOGICAL OXIDATIONS. ANIMAL HEAT REGULATION

The chief source of energy for all forms of life is the oxidation of food substances such as carbohydrates, fats and fatty acids. These, together with intermediate products of metabolism, are called **metabolites**. Other chemical processes, hydrolysis for example, furnish very little energy. Some vital processes, such as syntheses, are endothermal, that is, require energy from some source other than the reacting substances. Endothermal reactions store up energy in a potential form in the products of the reaction. Photosynthesis is the outstanding example of physiological endothermal reactions. But life processes require exothermal reactions, that is, those which liberate energy from its potential forms in physiological fuels. Such reactions are required in order that energy shall be available for heat production, growth, chemical syntheses, muscular and other contractions, secretion, the maintenance and repair of living structures and other protoplasmic activities. Physiological oxidations are, quantitatively, the most important exothermal reactions of living matter. The rate of oxidation in any tissue is, in general, a measure of its vital activity. The complete cessation of oxidation is the sign of death.

The Nature of Oxidation.—Typical oxidative reactions are those in which oxygen unites with something to form a true chemical union. But many other reactions are also called oxidations. Any reaction which removes hydrogen from a compound is conventionally called an oxidation, even though the hydrogen may not unite with oxygen but combines with something else. The change of a ferrous compound to a ferric one and all similar reactions which involve an increase of valence are called oxidation. Indeed, if the conventional idea of valence is accepted, every oxidation may be regarded as raising the valence of something. This means an increase of electrical charge and implies a corresponding decrease in the charge or electrical potential of something else. Oxygen itself shows two principal valences, 2 and 4. In many cases, its ability to oxidize is due to its ability to change from a valence of 4 to one of 2. It is theoretically possible that oxygen never oxidizes anything, except it is itself reduced in valence. Inasmuch as valences are merely positive or negative charges, oxidative reactions are really electrical phenomena in a certain sense. The electrical character of reactions between electrolytes has long been recognized but a similar understanding of oxidative reac-

tions has been of slow and recent growth. This was possible only after knowledge of the electronic structure of matter had developed. But in the light of the idea that oxidation requires the transfer of electrical charges from one compound to another, certain aspects of oxidation become intelligible.

The chief of these aspects is the fact that an oxidation is possible only when something is simultaneously reduced. In many cases, oxygen itself is the thing which is to be regarded as reduced. It loses its power to cause certain oxidations. In other words, it loses in the intensity of what is called its oxidation potential. For example, oxygen in the form of CO_2 has lost the power for oxidation that it possesses when in the form of O_2 . CO_2 may be regarded as a compound containing reduced oxygen or as one containing oxidized carbon. A further illustration of this principle is found in reactions of dehydrogenation. When an alcohol is oxidized (dehydrogenated) to an aldehyde, with palladium as a catalyst, the reaction,



(A primary alcohol. (An aldehyde formed
R stands for any by oxidation of
organic radical) alcohol)

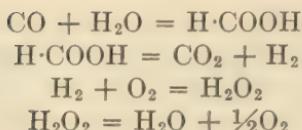
may go from left to right if some substance capable of taking up hydrogen, that is, a reducible substance, is present. Probably the H_2 is first taken up by the palladium and may then combine with atmospheric O_2 . But the reaction can go on even when no free oxygen is available, provided the hydrogen is cleared out of the way by some reducible substance which combines with it. Similarly, when sugar is oxidized in a solution of copper hydroxide and potassium hydroxide, the copper is reduced from the cupric to the cuprous condition. It loses one of its free electrical charges. It can no longer oxidize sugar so that it has lost in oxidation potential; but it can still oxidize H_2 to H_2O or CO to CO_2 , being itself reduced to metallic copper, so that it possesses a higher oxidation potential in the cuprous condition than it has in the metallic condition.

Comparison between Physiological and Other Oxidations.—The oxidative reactions within living cells cannot be observed in minute detail because the manipulations necessary for such observations generally destroy the structure of living matter and thus modify all its activities. Physiological oxidations have been investigated largely by the use of tissue extracts, macerated tissues and, more particularly, by study of the oxidations of physiological substances under the influence of chemical reagents such as peroxides. In view of these limitations of method, it is important to consider the question whether or not physiological oxidations are comparable to those observed *in vitro*. The great pioneer in the study of oxidation, Lavoisier, was clearly convinced that oxidations

in the animal body were very similar to those in non-living matter. This idea has been seriously questioned, but the accumulated evidence from modern researches has increasingly shown that certain oxidative reactions obtained *in vitro* are closely analogous to some that occur *in vivo*.

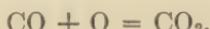
One general aspect of physiological oxidations makes it difficult to accept the idea that these reactions are comparable to other oxidations. Physiological processes occur at temperatures that are conspicuously lower than those required for the oxidation of the common physiological fuels in the absence of living matter. Sugar and fats, for example, are oxidized in living things at temperatures rarely above 40°C.; but require much higher temperatures for their oxidation by burning or by typical laboratory procedures. Another way of stating this fact is to say that nearly all of the foods of plants and animals are not "autoxidizable," that is they do not oxidize spontaneously, at room temperature, in the presence of air (O_2). Yet the facts of respiration indicate that O_2 is being continually used for oxidation by the living cells. This difficulty has been met, in part, by a study of the effects of catalysis upon oxidation. It is known that a substance that is not "autoxidizable" can be oxidized *in vitro* by the aid of a suitable catalyst and at much lower temperatures than would be required without the catalyst.

Certain analogies between physiological and other oxidations have been clearly shown. The presence of water at some stage of every oxidation is apparently necessary whether it occurs in living matter or elsewhere. For example, the oxidative processes that accompany plant activities are stopped by drying. A dried seed carries on practically no respiratory exchange until it is moistened. It then begins to give off CO_2 which is a sign of oxidation. Correspondingly, so simple an oxidation as that of carbon monoxide appears to require water. A completely dry mixture of carbon monoxide and oxygen cannot be made to explode by means of an electric spark, but this mixture readily explodes after the introduction of the slightest traces of moisture. Moreover, the rate at which this oxidation occurs increases, within certain limits, with increase in the small amounts of water vapor present. On account of the electrolytic dissociation theory, this and similar observations suggested, very naturally, that water was needed for oxidations in order to produce ions upon which free electrical charges would be available to further chemical reaction. But further investigations have indicated, as was earlier suspected, that water takes a more direct part in oxidation processes. In some cases at least, water actually furnishes the oxygen and is itself reduced so as to liberate hydrogen. The latter unites with atmospheric oxygen. Through the work of several investigators, the following scheme has been shown to be the probable representation of the course of the reaction in which carbon monoxide is oxidized with the aid of catalysts, such as palladium black.



This scheme represents carbon monoxide as uniting with water to produce formic acid which breaks down to liberate carbon dioxide and hydrogen. The latter unites with molecular oxygen to form hydrogen peroxide which, in turn, breaks down to liberate water and oxygen. These equations are based upon experiments in which the intermediate products, formic acid, hydrogen and hydrogen peroxide were actually identified and proved to be intermediate products of this reaction. The scheme represents the oxidation of carbon monoxide as due to water and not to oxygen. This idea is substantiated by the established experimental fact that carbon monoxide and water vapor can react, in the presence of palladium black as a catalyst, to form carbon dioxide and hydrogen *in the complete absence of free oxygen*. The reaction cannot go very far, of course unless some substance that is able to unite with the hydrogen is present. When such a substance is available, this reaction proceeds readily. It occurs at room temperature in spite of the fact that carbon monoxide is not "autoxidisable." Other oxidations have been shown to occur similarly, so that the participation of water in catalyzed reactions may be as common *in vitro* as it appears to be *in vivo*.

Another analogy between physiological and other oxidations is found in the way in which both of them occur in stages, that is, in a series of linked reactions. This has long been appreciated in the case of physiological reactions. The recognition of intermediary products of linked reactions has afforded some insight into the chemical reactions actually occurring in living matter. For example, the production of lactic acid in animals has been shown to be due to the incomplete oxidation of carbohydrate. Yet this tendency of chemical reactions to occur in stages is not characteristic of living matter alone, but is found to prevail quite generally among reactions that are as supposedly simple as the one that is summarized by the equation:



Even this "simple" reaction is really a series of four reactions which though they occur simultaneously and interdependently can nevertheless be distinguished from one another.

There are reasons, then, for assuming that the study of oxidations *in vitro* throws some light on the nature of physiological oxidation: (1) Many oxidations *in vitro* are found to be dependent upon the presence of water just as physiological oxidations appear to be. (2) The linking of reactions so as to constitute an oxidation is shown to be characteristic

of so-called simple oxidations as it is of physiological ones. (3) Oxidations can be artificially catalyzed so that they proceed at temperatures compatible with life even though, without a catalyst, these same reactions would not occur except at comparatively high temperatures. Catalysts commonly occur in preparations made from tissues and are probably present in all active cells.

Catalytic Activations of Oxygen.—The catalysts concerned with general physiological oxidation have been called the **oxidases**. This distinguishes them from more special oxidizing enzymes to be described later. But this terminology is somewhat misleading because these enzymes probably never catalyze the actual reactions which oxidize physiological fuels, but promote oxidations in an indirect way by rendering oxygen active so that it attacks the material to be oxidized.

Active oxygen is commonly defined as nascent oxygen. It is oxygen at the moment it is liberated from its compounds or from the molecular state. Ozone (O_3) is especially effective as an oxidizing agent because it is comparatively unstable and readily liberates nascent oxygen. The oxidation of certain physiological substances has long been known to occur *in vitro* in the presence of ozone. This fact throws little light on the nature of physiological oxidations because ozone does not occur in living matter and is even highly toxic to cells. But closely related to ozone are the peroxides, such as hydrogen peroxide (H_2O_2), the metallic peroxides (Na_2O_2 , BaO_2 , etc.) and the organic peroxides ($R—O—O—R$, in which R is any organic radical). The peroxides are strong oxidizing agents. They are all characterized by having oxygen atoms so arranged as to be combined with each other in an unstable condition. The oxidation of certain biological substances such as vitamins, fatty acids and glucose, have been effected *in vitro* at room temperature by the use of hydrogen peroxide. In the case of fatty acids and glucose a suitable catalyst is required for this reaction.

The Bach-Engler Theory of Oxidation.—Peroxides have not been actually identified in living cells and their presence in detectable amount is indeed unlikely as they are generally toxic. But much indirect evidence points to the idea that they may occur momentarily as intermediate products of oxidation in cells. This idea is based on extensive experimental work and was formulated independently by Bach and by Engler. It is generally known as the Bach-Engler theory of oxidation. It postulates that O_2 reacts with some protoplasmic substance to form a peroxide which then breaks down to liberate active oxygen, which oxidizes metabolites. The hypothetical substance which is supposed to produce the peroxide is called an **oxygenase** and the one which liberates oxygen is called a **peroxidase**. These names imply that the respective substances are enzymes. This is in agreement with the observation that they appear to act on indefinite amounts of material and fail to act

after heating. Evidence for their existence is found in experiments such as the following.

The flesh of the potato turns black on exposure to the air. This is evidence of oxidation because the black substance is known to be produced by artificial oxidation of the amino-acid tyrosine and certain tyrosine compounds such as occur in cell products. A still more striking demonstration is obtained with guaiacolic acid or with extracts of the tree gum, called guaiac, which contains guaiacolic acid. This substance is used because it readily oxidizes to yield a bright blue compound but is not "autoxidizable." Placed upon freshly exposed surfaces of potato or mixed with potato scrapings, guaiacolic acid turns blue. Similar results are obtained when this acid is brought into contact with numerous substances of plant and animal origin. These reactions do not occur when air (O_2) is excluded. Such experiments of themselves do not give evidence to show that peroxide formation is a part of the oxidative process. But Bach obtained such evidence in the following way. He first showed that tyrosine is rapidly blackened by fresh potato juice in the presence of air. He then treated some potato juice with alcohol so as to obtain a precipitate. This was dissolved in water and found to be without any power to oxidize tyrosine. But when H_2O_2 was added to this mixture the blackening occurred. This at least shows the H_2O_2 can take the place of something furnished by the potato. It also shows that the precipitate produced by alcohol can render H_2O_2 capable of yielding active oxygen. This is the case because tyrosin is not oxidized by H_2O_2 alone. In terms of the Bach-Engler theory, the precipitate contains peroxidase but no oxygenase; while the cells and the fresh juice of the potato contain both of these substances. Certain tissues of plants and animals appear to contain peroxidase without any oxygenase. The horseradish, for example, yields a juice which turns guaiacolic acid blue if H_2O_2 is added to it, but does not cause this oxidation without a peroxide.

Is There an Oxygenase?—Although the enzyme characteristics of peroxidase seem well established, those of oxygenase have not been definitely shown. The mere fact that tissues and tissue extracts do not oxidize in the absence of O_2 indicates that peroxides are not stored in the cell and that something represented by the name of oxygenase must be present in the cell in order that things which are not autoxidizable shall be oxidized. But the so-called oxygenase need not necessarily be an enzyme. Many reactions are known to occur between O_2 and "autoxidizable" substances with the formation of peroxides which are thus made available to oxidize some substance that is not "autoxidizable." For example, the oxidation of oil of turpentine by atmospheric oxygen can cause the simultaneous oxidation of dyes, such as indigo blue, which are not "autoxidizable" but which are oxidized by peroxides. Turpentine, exposed to air, contains something of a peroxide nature. If cells

contain some autoxidizable substance which reacts with O_2 to form a peroxide, perhaps this substance would be permanently oxidized itself by the reaction and therefore would not be a true catalyst. This reaction would go on concurrently with those which caused the breakdown of the peroxide by peroxidase and the oxidation of cell metabolites by active O_2 . The three types of reactions would thus constitute a series of linked ones or what is often called a coupled reaction. If on the other hand, the autoxidizable substance were reduced to its original condition it would be a true catalyst. It is impossible to decide, in the light of present knowledge, whether there is an oxygenase or whether an autoxidizable substance is itself oxidized to supply any peroxides that may arise during physiological oxidations. As will be seen from the discussion below, the Bach-Engler theory is not the only possible one to account for physiological oxidation.

The Nature of Peroxidase.—Certain salts of iron, manganese and copper, metals which readily exist in two states of valency, have the power of liberating active oxygen from peroxides so as to further the oxidation of substances that are not autoxidizable. One or more of these metals appears to be present in some organic combinations in every actively oxidizing cell. The use of manganese for the preparation of an artificial oxidizing enzyme suggests that this metal may be of significance in some physiological oxidations. Warburg has shown that cytolized sea urchin eggs show a marked increase in O_2 consumption when iron salts are added. Moreover, iron is known to further the oxidation of certain lipoids, such as lecithin. Warburg obtained further evidence of the significance of iron in oxidation by studying the effect of potassium cyanide upon cells. He found that a certain concentration of potassium cyanide entirely stops oxidation in various kinds of cells. Eggs and heart muscle have been particularly studied. The cells recover their oxidative powers after the cyanide has been washed away by a suitable solution. A significant fact in connection with the nature of oxidative catalysts is that the amount of cyanide just sufficient to stop oxidation is the quantity which would be required to combine with all the iron of the cells as determined by analysis of their ash. These and some other experimental observations have led to the hypothesis that peroxidases contain salts of a metal, probably of iron or of manganese, in some colloidal complex.

Catalase.—In addition to peroxidase, one other enzyme has been described as concerned with general physiological oxidation. This is known as catalase. It has the power to cause the liberation of oxygen from hydrogen peroxide, but the oxygen so liberated is in the inactive form of molecular O_2 . As such it produces no oxidative effects. Catalase is distinctly different from peroxidases for the latter do liberate oxygen from hydrogen peroxide in an active form. Catalase has a very wide

distribution. It has been detected in numerous different organs of very many species of plants and animals. On account of this wide distribution, it has been supposed by some writers to be concerned with the process of oxidation. Attempts have been made to show that the catalase content of different tissues is proportional to the intensity of their normal oxidative processes. Quantitative estimations of the catalase content of tissues are made by allowing a given amount of the tissue to act upon a standardized solution of hydrogen peroxide. It is important that in such tests the hydrogen ion concentration and all other conditions surrounding the experiment should be very carefully controlled. The O_2 evolved from H_2O_2 is collected and measured. From the rate at which oxygen is given off the comparative catalase content can be estimated. Such observations do not show a parallel between the abundance of catalase and the normal oxidizing powers of tissues. It has been shown, for example, that cells which are in stages of rapid growth and development, during which their rate of oxidation is especially high, do not have a correspondingly high catalase content. Yet it is hard to believe that this substance should be almost universally present in living structures, unless it is of some real use. It has not been shown to have an effect on the oxidation of any cell metabolite. The only reaction which it is known to catalyze is the decomposition of hydrogen peroxide. The most acceptable theory as to its usefulness is one which supposes that catalase serves to protect cells from the presence of any peroxide not used by physiological oxidations. If this theory is the right one, it lends added weight to the hypothesis that peroxides arise during the course of physiological oxidations.

Specific Oxidizing Enzymes.—Certain enzymes concerned with oxidation are more specific than those described above. One of these is **tyrosinase**. As this name implies, it is concerned with the oxidation of tyrosine and related compounds. It has a widespread occurrence. It was first found in fungi but has been recognized in certain animal tissues and is frequently found in structures of the higher plants. The demonstration of its occurrence in the potato was described above. The dark colored or black substances formed by its action are complex. Their structure is not established. They include some of the natural pigments of plants and animals. The nature of the reaction catalyzed by tyrosinase is not simple. Tyrosine is known to be first reduced and then oxidized.

Another specific oxidizing enzyme is the one called **xanthine-oxidase**. It oxidizes xanthine to uric acid. Suspensions of macerated animal tissues effect this change with the aid of atmospheric oxygen, but fail to do it when oxygen is excluded. This substance shows all the characteristics of an enzyme. It is not uniformly distributed among the organs of animals of different species. It is most commonly present in the liver

and spleen. Presumably, it plays a rôle in the disposal of the so-called purine bases which arise in the animal through the hydrolytic breakdown of nucleoproteins. Inasmuch as each of these purine bases is convertible, in the animal body, to xanthine, xanthine-oxidase aids in disposing of all of them. This same enzyme may also catalyze the oxidation of hypoxanthine to xanthine.

A third specific oxidase is **uricase**. This catalyzes a reaction in which uric acid undergoes an oxidative decomposition to form several products among which, allantoine is the most prominent. This enzyme occurs in some of the organs of numerous different animals but not necessarily in the same organs of animals of different species. It disposes of any excess of uric acid, converting it into more soluble and possibly more easily excreted substances. Curiously enough, this enzyme does not occur in any human tissues although it has been recognized in the tissues of numerous mammals and other laboratory animals. The enzyme is also lacking in the tissues of certain anthropoid apes. Although commonly found in the tissues of dogs, this enzyme is lacking in the liver of the Dalmatian coach hound. This animal accordingly excretes uric acid in amounts comparable to that found in human urine.

A specific oxidizing enzyme called **laccase** is found in the juices of the lac tree and in certain fungi. This enzyme causes the oxidation of certain phenol derivatives to products that vary according to the derivative which forms the substrate. This enzyme has received considerable attention from investigators because it has been shown to contain manganese. An artificial laccase has been prepared from a mixture of manganese hydroxide and a vegetable gum.

Another specific oxidizing enzyme is the one called **luciferase**. This catalyzes a reaction in which a specific substrate, called **luciferin**, is oxidized. During this reaction, light is given off. It is therefore called a *photogenic reaction*. It is the source of the light emitted by certain species of animals. As this or some very similar reaction probably accounts for light production wherever it occurs in living things, it deserves some discussion.

Oxidative Light Production.—This phenomenon occurs, among plants, in many species of bacteria, including bacillus and coccus forms, and in several species of mushrooms. It occurs, among animals, in a number of species of protozoa, in various hydroids, in some jelly fishes, in sea pens and certain other actinians, in the etenophors, in a few of the bryozoa, in many kinds of worms, in a few species of the brittle star fishes, in crustacea, including numerous ostracods and copepods, in some of the myriapods, in numerous insects, such as fireflies and certain beetles, in many species of molluses, in some of the lower chordates and in fishes, including representatives of both elasmobranchs and teleosts. The occurrence of light production among living things is seen, even from this abbreviated

list, to be very haphazard and without apparent relationship to taxonomy. Although there are both marine and land forms that are luminous, no fresh-water light-producing forms have been found.

In many cases, light production has been attributed, erroneously, to higher plants and animals because infection with light-producing bacteria has made them appear to be luminous. The glowing of decayed wood, of seaweeds, of meat, of animal wounds and of fish flesh can often be observed in the dark, but is due merely to bacterial infection.

The luminous structures of protozoa are granules or globules scattered through the protoplasm. In multicellular animals, specialized luminous organs produce the light. In some cases, for example in the fireflies, the light is produced within these organs under nervous control and flashes in response to excitation. In lower organisms, the light-producing organs are glands. They secrete luminous material which is poured out, in many cases, over the surface of the body so that the entire animal is seen to glow in the dark.

The chemistry of light production has been studied by numerous investigators. E. Newton Harvey has extensively investigated light production in animals, especially *Cypridina*, an ostracod, and the fireflies. The work of DuBois on *Pholas*, a lamellibranch, has also been fundamental. These and some other researches have shown that luminous organs of animals, either fresh or dried, can be treated with boiling water to yield an extract which will not luminesce on exposure to air but which contains something necessary for bioluminescence and able to produce light in the presence of unboiled extracts of luminous organs, provided that oxygen, even though in low concentration, is dissolved in the extract. On mixing these two solutions, bluish- or greenish-yellow light glows throughout the mixture and is very striking when seen in a dark room.

Luciferin, as mentioned above, is the name given to this thermostable (not destroyed by boiling) substance. It appears to vary in composition according to the source from which it is prepared. At least there are marked differences in the behavior of luciferin as obtained from *Pholas* and *Cypridina*, respectively. All luciferins show colloidal behavior, salting out reactions and precipitation by numerous reagents so as to indicate that luciferins may be proteins. Harvey has shown that *Cypridina* luciferin is similar to some of the proteoses, so far as certain of its reactions are concerned, but it is soluble in absolute alcohol, is not digested by trypsin and does not give a biuret test. If it is a protein it is not a typical one.

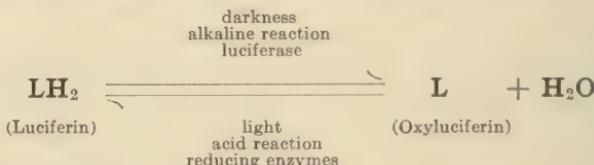
Luciferase, the thermolabile component of the bioluminescent reaction, has the chemical behavior of albumins. Luciferase is prepared, most easily, by merely extracting luminous organs with cold water and letting the extract remain exposed to air until it ceases to give light because all of its luciferin is oxidized. Addition of luciferin, prepared by hot water

extraction, then causes light to flash up again. Luciferase has been prepared in a state of partial purification by salting out with $(\text{NH}_4)_2\text{SO}_4$, by precipitation with alcohol and by other manipulations applicable to albumins. Luciferase, like luciferin, is different according to its source, although it is not absolutely specific. It will cause light production with luciferin from related species. But luciferase of the *Photuris* (firefly) does not cause luminescence with luciferin of *Cypridina*, nor does luciferase of the latter luminesce with luciferin of the firefly. The enzyme character of luciferase is indicated by its sensitiveness to changes in temperature, C_H , etc. and by the fact that a comparatively small volume of luciferase solution will cause complete oxidation of a large amount of luciferin. Harvey estimates that one part of luciferase can act on 10,000 parts of luciferin. Luciferase is destroyed, however, during its activity, so that it is not proved to be a true catalyst in the strict sense of the word. But in view of the possibility that luciferase may be destroyed by a subsidiary reaction rather than the one which it appears to catalyze, its enzyme character is not disproved by this circumstance.

The oxygen concentration required for the reaction is not high. Harvey found that light production was very good in preparations from *Cypridina* when hydrogen, containing only 0.4 per cent of oxygen was bubbled through them. This gave an O_2 tension of approximately 3 mm. Hg. The minimal O_2 concentration required is so low as to have caused earlier investigators to state that the photogenic reaction could occur without oxygen. But yeast and many reducing bacteria can exhaust oxygen in a solution so as to prevent light production completely. When the solution is agitated to cause oxygen to dissolve in it, light flashes up again. Harvey found that O_2 tensions between 0 and 7 mm. Hg. caused increase of luminescence with increase of oxygen; but at 7 mm., the light appeared to be just as bright as when the solution was saturated with oxygen at atmospheric tension (152 mm. Hg.).

The nature of the reaction, though clearly oxidative, does not appear to be such as to produce CO_2 . This is indicated, according to Harvey, by the absence of any increase in the C_H of the solution during the reaction. It is different from most oxidations in another respect. It does not give off any detectable amount of heat. Delicate measurements with a thermocouple led Harvey to conclude that any small heat production that may occur is certainly less than 0.01 Calorie per gram of luciferin oxidized. In the production of light without heat, living organisms are unique. All other known means of light production involve the liberation of much heat and are correspondingly wasteful of energy when, as in ordinary artificial lighting, light only is required. Light is given off by the luciferin-luciferase reaction only while the oxidation is in progress. The relative intensity of the light is determined by the rate of oxidation. If, for example, a given luciferin solution is oxidized in one case in the pres-

ence of a comparatively low concentration of luciferase and in another case in the presence of more concentrated luciferase, the second one will give the greater light intensity and will be through with its light production and become dark sooner than the other. Rise of temperature, up to a certain optimum, also increases the light intensity by speeding up the reaction. The reaction is reversible under certain circumstances. The oxidation product, the so-called **oxyluciferin**, can be reduced. This is accomplished by the action of reducing bacteria and by several different reducing reagents, such as nascent hydrogen, ammonium sulphide, and reducing enzymes of animal tissue extracts. When so reduced, luciferin can be reoxidized in the presence of oxygen and luciferase. Alkaline reaction of the solution favors the oxidation and an acid reaction favors the reduction. Darkness favors the oxidation and light the reduction. Harvey has suggested that oxidation of luciferin may be a dehydrogenation and reduction a recombination with hydrogen. He represents this idea in the following scheme:



This reaction gives off light in going from left to right.

Photogenic reactions, between substances of known composition, are not uncommon. The oxidation of pyrogallic acid by ozone, hydrogen peroxide or other oxidizing agents and the oxidation of phosphorus are good examples. Many such typically photogenic reactions go on at temperatures below 100C. and are clearly distinguished from reactions, such as burning of fuels, in which some substance is made to glow by mere heating. But none of the photogenic reactions between laboratory reagents can compare with the luciferin-luciferase reaction in producing light without heat, for all of them give off much more energy as heat than as light.

Hydrogen Acceptors—Reduction actually occurs in cells as is demonstrated in a number of ways. One of these is the injection of a dye which becomes colorless when reduced but reversibly regains its color upon oxidation. Alizarin blue and indophenol blue were employed to demonstrate reduction in experiments made by Ehrlich. These dyes penetrate cells without killing them, thus effecting what is called intravital staining. When an animal, injected with one of these dyes, is killed some of the organs are found to be blue and others not so colored; but the latter become blue upon suitable exposure to air, thus showing that they contain the dye in reduced form. Those in which the dye remains oxidized have a higher oxidation potential than do those which reduce it. The

reduction of these substances is not surprising because every oxidization, even those in living matter is accompanied by a reduction of something. In case oxygen is not present in readily reducible form some other substance may be reduced.

It is conceivable that metabolites enter into the process of being oxidized by giving off hydrogen to some easily reducible substance (a hydrogen acceptor) which is thus reduced but which gives off the hydrogen so readily and in such an active form that it combines with oxygen to produce hydrogen peroxide which then breaks down to liberate water and one-half the oxygen which entered into the reaction. This would be analogous to the oxidation of CO as described above. Experimental evidence in support of this conception has been obtained. Some of it follows.

1. The Schardinger reaction, which is used, practically, to distinguish fresh milk from that which has been heated, shows oxidation in the absence of oxygen. Fresh milk is treated with an aldehyde and methylene blue or indigo blue. The aldehyde is oxidized and the dye is reduced to a colorless compound. Fresh milk has very little reducing power unless the aldehyde is added. Heated milk fails to reduce (decolorize) these dyes either with or without the presence of aldehyde. The reduction appears to be due to an enzyme which causes the transfer of hydrogen from the aldehyde to the dye. The latter acts as an acceptor of the hydrogen.

2. Cannizzaro's reaction gives similar evidence. In this reaction, two molecules of an aldehyde react with water to form one molecule of an alcohol and one of an acid.



(An aldehyde. R stands for (A primary alcohol formed by (An acid formed by oxidation of any organic radical) reduction of aldehyde)

In this case one molecule of aldehyde acts as a hydrogen acceptor and another is oxidized. The reaction is markedly hastened by the presence of macerated liver tissue or by extracts of some other organs. This acceleration is apparently due to an enzyme. At any rate, boiled tissues do not accelerate the reaction.

3. The reduction of methylene blue by animal tissues (liver, lung, spleen, kidney or thymus) gives further evidence. This reaction was investigated by Bach. He found that although suspensions of macerated liver tissue in water can rapidly reduce methylene blue, filtered extracts of liver lack this power. But when an aldehyde is added to the filtered extract it does reduce the dye. This reducing power of tissues is due to something which behaves in every way like an enzyme. Bach precipitated it from filtered liver extracts by the use of alcohol. The precipitated material, dissolved in dilute NaHCO_3 , reduced methylene blue in the presence of an aldehyde. These experiments show, as in the case

of those just described, that the dye acts as a hydrogen acceptor and the aldehyde is oxidized. The additional fact, shown by Bach's experiments, is that the liver furnishes something which can be oxidized in the presence of the hydrogen acceptor. This oxidizable substance is present in unfiltered, macerated liver preparations but is lacking in filtered liver extracts. In the latter, its role must be filled by some oxidizable substance, such as aldehyde, before the dye can be appreciably reduced. This gives a clear cut demonstration of the close relationship that exists between processes of oxidation and reduction.

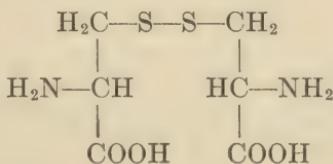
4. The reduction of nitrates to nitrites is accomplished by tissues and tissue extracts of animals and plants. In this case, also, the presence of some easily oxidized substance, such as an aldehyde, is required. This reaction also appears to be due to an enzyme. In the case of the liver, it may be the same one which causes the reduction of methylene blue; but Bach showed that potato juice effected the reduction of nitrates under conditions which did not permit of the reduction of the dye so that there may be a specific enzyme which causes oxidation of aldehydes with concurrent reduction of nitrates. In any case, the nitrate acts as a hydrogen acceptor.

5. Oxidation of certain tissue metabolites has been obtained *in vitro* without the aid of oxygen. In these reactions, studied especially by Wieland, palladium is used as a catalyst and causes the dehydrogenation of glucose, lactic acid and other substances which are known to oxidize as typical tissue metabolites, being intermediary products of the oxidation of typical foods of cells. Hydrogen is actually liberated by the palladium; but the reaction is impeded, as is to be expected, unless some substance is present to act as a hydrogen acceptor. Shaking the reacting mixture with air enables oxygen to play this role, so that the oxidation goes on to a greater extent than it does in the absence of oxygen. The products formed are identical in several cases with those obtained during physiological oxidations. Glucose yields $\text{CO}_2 + \text{H}_2\text{O}$, lactic acid is changed to pyruvic acid and the latter to acetic acid and CO_2 . These are reactions known to occur in living matter. These experiments show that such oxidations can be started by the process of dehydrogenation and that oxygen is required merely to combine with the hydrogen.

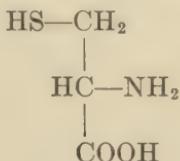
6. The acetic acid-producing fermentation caused by certain bacteria furnishes an especially striking demonstration of dehydrogenation as a means of causing oxidation. Wieland has shown that either the living or the dead bodies of these bacteria can oxidize alcohol to acetic acid in the complete absence of oxygen, provided some hydrogen acceptor is present. Methylene blue was one of the several reducible substances which Wieland found to be effective as acceptors. He was able to show that, as this dye was reduced, alcohol was oxidized to acetic acid in direct proportion to the extent of the reduction.

7. Frogs have been found to give off CO_2 in an atmosphere of pure nitrogen. The amounts given off are in excess of that which might be present in the tissues before exposure to nitrogen. This shows that oxidation of metabolites can occur in tissues of a cold-blooded animal even in the complete absence of free oxygen. This is probably explained by the comparative abundance of the oxygen-rich compound, glycogen, in such tissues. Warm-blooded animals do not have an abundance of glycogen in most of their tissues and are correspondingly sensitive to oxygen deprivation. Life without free oxygen is the normal state of many organisms, the anaerobic forms, which are described below (p. 600).

8. Finally, a substance capable of acting as a hydrogen acceptor has been isolated from animal and plant tissues. This notable result was obtained by Hopkins. He has named this substance **glutathione**. It is constituted of two amino-acids, cystine and glutamic acid. It owes its remarkable properties to the sulphur of cystine. This amino-acid has the formula,



It easily splits, in the presence of reducing agents, to form two molecules of cysteine,



thereby taking up two atoms of hydrogen. Cysteine does not oxidize spontaneously to cystine, but reduced glutathione does. In other words, glutathione is autoxidizable in a readily reversible reaction which may be pictured in its essentials, thus:



R is used to represent the part of the cysteine grouping not otherwise shown and also the glutamic acid radical. This reaction represents the behavior of glutathione as a hydrogen acceptor; but read from right to left, the reaction suggests its possible rôle in oxidations, in that the H_2 could combine with oxygen leaving the substance which donated the hydrogen in an oxidized (dehydrogenated) condition.

The possibility that some sulphur compound enters into physiological oxidation had been suggested before Hopkins discovery. Yeast

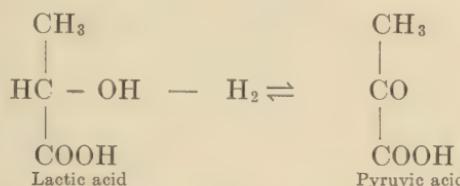
cells and many animal tissues have long been known to contain some substance which readily gives up H_2 to form H_2S with powdered sulphur. This and other reactions in which tissues give up H_2 have been shown to be related to the presence of a labile $-SH$ group in some cell constituent.

This relationship was established by the use of Mörner's delicate nitroprusside test for cysteine, which in the presence of ammonium sulphate and ammonia gives a brilliant purple color with sodium nitroprusside. This test is not specific for cysteine. Other substances, which contain the $-SH$ group or which, for some other reason are able to reduce the nitroprusside molecule, give the test. But substances of this sort probably do not occur in tissues and the intensity of the nitroprusside test color as given by specific types of cells, is known to be proportional to the amount of reduced sulphur present in them. Proteins containing cystine and cysteine itself do not give this reaction nor do the cystine groups of proteins reduce to cysteine so as to enable proteins to give the test. These facts all point to the occurrence of cysteine in cells, in some form other than a protein. Hopkins has shown that this form is reduced glutathione.

It occurs in the cells in a "free" condition, free in the sense that it can be washed away from macerated tissues by water. It appears to be widely distributed. Inasmuch as reduced glutathione gives the nitroprusside test, Hopkins has used it to obtain evidence of the occurrence of this substance in actively growing plant tissues, in all bacteria that have been examined, in yeast, in muscles of the earth-worm, the oyster, the blowfly, the lobster, the skate, the cod and the frog and in all the organs of mammals. It seems to occur more abundantly in typically active tissues than in those which do not metabolize intensely. It is abundant in muscle and liver but lacking in connective tissue. It is lacking in blood plasma and serum. It is richly present in the chick embryo even when it is only one day old, but is not found in the egg. Positive results with the nitroprusside test do not necessarily prove that glutathione is present, but Hopkins has shown that glutathione preparations made from muscle, from liver and from yeast are identical and has obtained some evidence to show that no other normal cell constituent gives the test. Glutathione occurs in small amounts, about 0.01 to 0.02 per cent of fresh tissues.

Hopkins showed that the purified substance can exert marked effects upon reduction when very small amounts of it (less than 0.1 per cent); are added to macerated tissue suspensions containing methylene blue. Its effects are markedly influenced by the reaction of the medium. At pH = 6.8, just on the acid side of neutral, it hinders reduction but at pH = 7.4, faintly alkaline, it favors reduction. This indicates that the formation of acid in tissues makes glutathione especially effective as an aid to oxidation because it then takes up hydrogen with great avidity, at least it

successfully competes with methylene blue in holding hydrogen. Glutathione is nevertheless autoxidizable even in acid solution. The production of lactic acid is apparently the first chemical reaction that results from excitation of muscle (p. 301). The subsequent oxidations, by which muscle recovers from the effects of excitation, should be furthered by the presence of glutathione. The lactic acid is probably changed to pyruvic acid in the first step toward oxidation.



This reaction clearly involves the removal of two atoms of hydrogen from the lactic acid. Glutathione could well serve as acceptor for the hydrogen. The reduction of glutathione is catalyzed by all fresh tissues and this effect shows every evidence of being due to an enzyme.

Wieland's Theory of Dehydrogenation.—The work of numerous investigators and especially that of Wieland led to what is called Wieland's theory of dehydrogenation. It postulates that some of the oxidations and reductions occurring in cells are due to an enzyme which causes the transfer of hydrogen from a metabolite to a hydrogen acceptor and that this is the only enzyme required for certain oxidations. This is possible because the acceptor itself is autoxidizable. Reducing enzymes had been named previously as reductase or hydrogen-transportase. Wieland proposed the more descriptive name **dehydrase**. He suggested that this enzyme, by catalyzing reduction, could cause oxidization, in some cases at least, without a peroxidase. It could also catalyze reactions in which water is split to cause both oxidation and reduction as in Cannizzaro's reaction. Such a process is called a hydrolytic-oxidative-reduction. The compounding of the three words is helpful because it calls attention to the coupling of three reactions, water splitting, oxidation and reduction, which go on so closely interlinked that they appear like one reaction. As such reactions frequently involve oxidation of an aldehyde, the enzyme which catalyzes them was named **aldehyde-mutase**. Wieland believes that this too is nothing but dehydrase.

The discovery of glutathione has given Wieland's theory a firmer basis than it previously had. Glutathione itself can hardly be regarded as an enzyme. In some ways, it does resemble a catalyst. It can function over and over again without apparently being used up. It is restored to its original condition after it has participated in an oxidative reduction. But it is thermostable and the reactions which reoxidize it, after it has

been reduced, are not necessarily coupled with those which it promotes. In this respect, it differs from an enzyme. Moreover, a catalyst which appears to be a true enzyme, dehydrase, is required to reduce glutathione. Hopkins speaks of it as co-enzyme to dehydrase.

Life without Free Oxygen.—The dehydrogenation theory helps to explain oxidation in which oxygen, as such, need not necessarily participate. That some organisms exist in the absence of any free oxygen has long been known. They are called anaerobic organisms and life without free oxygen is called **anoxybiosis** or **anaerobiosis**. As early as 1855 Bunge showed that the intestinal worm, *ascaris*, retains the power of active motility during several days in an oxygen-free salt solution and meanwhile gives off CO₂. In the intestine, O₂ tension is normally 0. These organisms cannot make use of oxygen when it is supplied to them. Weinland showed that glycogen was extensively used by these animals. They normally contain much of this carbohydrate which may compose as much as one-third of the solids of their bodies. When they are deprived of food, this glycogen is markedly depleted. Earthworms have also been shown to use glycogen during their anaerobic processes of oxidation. When they are supplied with air, after a period of anoxybiosis they use abnormally large amounts of O₂ in proportion to the CO₂ which they give off. This indicates that they store away oxygen-rich compounds when they have the opportunity. Glycogen is such a compound and is known to increase in their bodies during periods of living in air. The rich glycogen store in the tissues of worms is doubtless a significant factor in fitting them for anaerobic existence.

Carbohydrates are not the only metabolites oxidized during anaerobic existence. Weinland showed that a macerated suspensin of fly larvae (puperae of *Calliphora*) were able to destroy fat in the absence of O₂, forming CO₂ and H₂. This reaction thus involved dehydrogenation.

Aside from the worms, such as the parasitic ones, multicellular animals do not normally live in an oxygen-free medium. Nevertheless, cold-blooded animals can exist and even effect some oxidation during considerable periods of oxygen deprivation. Frogs have been kept alive 24 hours or even longer in an atmosphere of pure nitrogen. The leech has been similarly kept 10 days. Snails have also been shown to survive prolonged oxygen deprivation.

Yeasts and many fungi are able to exist without free oxygen. Under anaerobic conditions, their metabolism is not the same as it is when air is present. Yeasts, for example, do not grow unless they have a supply of free oxygen although they cause fermentation of sugar to alcohol and CO₂ either with or without oxygen. Pasteur showed this. He also found that the mould, *Mucor racemosus*, oxidizes sugar to CO₂ + H₂O in the presence of O₂; but under anaerobic conditions, this mould causes the fermentation of sugar to alcohol and CO₂.

These yeasts and fungi and also many kinds of bacteria are examples of what are called **facultative anaerobes**. This term refers to their ability to exist either with or without free oxygen. Certain bacteria, however, are killed or at least changed to an inactive spore form by even very low concentrations of oxygen. Such bacteria are called **obligatory anaerobes**. Most of them are putrefactive organisms. All typical putrefactions are anaerobic, whether produced by facultative or obligatory anaerobes. The products of protein putrefaction were described in connection with bacterial action in the intestines.

Energy Requirements in Anaerobiosis.—Organisms depend largely upon carbohydrates during anaerobic life. When, as in the case of yeast, alcohol and CO_2 are produced, these compounds possess less potential energy than does the carbohydrate which was fermented. The difference between these two values represents the energy which the anaerobic organism can use for its life processes. This is not a large quantity. Glucose, decomposed to alcohol and CO_2 , gives less than 5 per cent of the energy which is liberated when complete oxidation to $\text{H}_2\text{O} + \text{CO}_2$ occurs. An organism must utilize a correspondingly large amount of sugar to obtain a given amount of energy by alcoholic fermentation. The quantity of sugar that can be fermented by yeast is surprisingly large, amounting to several times the mass of the yeast in the course of a few hours. As a rule, anaerobic organisms do not require much energy in comparison with that needed by aerobic forms. Yeast requires energy for growth and maintenance of cell structure. It also produces heat but does not ordinarily need to raise its temperature above that of the environment in order to maintain activity. The same is true of bacteria. Parasitic worms live in an environment which protects them from temperature changes. They require energy, however, for muscular contraction and other tissue functions. But on the whole, anaerobic forms have low energy requirements and in any case, these can be met by the mildly exothermic reactions that occur in their tissues. In such reactions, carbohydrates play a leading part.

Indispensability of Carbohydrates.—The oxidation of carbohydrates is an indispensable part of normal metabolism in many organisms. It may be that all organisms have to make use of carbohydrate in order to attain their maximal activity. Green plants synthesize carbohydrates which are utilized in plant oxidative metabolism. Fungi do not attain maximal growth unless carbohydrate is supplied to them. Bacteria require either carbohydrate material or the closely related polyhydric alcohols, such as sorbitol or glycerol, in order to maintain vigorous growth. All animal forms, even the amoeba, can carry on only a deranged and abnormal metabolism in the absence of a carbohydrate food supply. The oxidation of carbohydrate has been studied more particularly in animals and especially in mammals, although many

aspects of carbohydrate utilization in bacteria and yeasts have been extensively investigated. The following account will deal chiefly with carbohydrate oxidation in mammals.

Carbohydrate Oxidation in Animals.—Energy production in muscles is dependent upon carbohydrate oxidation. Although fats and the organic acids derived from the breakdown of amino-acids are also utilized in the animal for energy production, they are not fully oxidized unless carbohydrate is simultaneously used. Moreover, there is some evidence to indicate that these substances are never completely oxidized, as such, in muscles but are first partially transformed, somewhere in the body, into carbohydrates. Muscles are the chief animal oxidizing organs in the sense that they oxidize more material than do all the other organs put together. The preference of the muscles for carbohydrate thus takes on a fundamental significance.

Carbohydrate Transformation to Glucose.—The carbohydrate which is oxidized in animals has been commonly stated to be glucose. This is the sugar normally present in the blood. All digestible polysaccharides and disaccharides are normally changed to monosaccharides before absorption from the intestine. The only exception to this rule occurs when an unusually high concentration of a soluble carbohydrate, such as a disaccharide or a dextrin, is present in the intestine. In this case, it appears in the blood. There is no conclusive evidence to show whether or not it is actually used. The monosaccharides, normally absorbed, consist chiefly of glucose. Galactose and fructose may also be present, usually because of lactose and saccharose of the food. In herbivores, pentoses are also to be reckoned with. They constitute a large part of the hydrolysis products of certain celluloses. All these monosaccharides, other than glucose, appear to be transformed into glucose. How does the animal effect this change? *In vitro*, such transformations have been produced only by conversion of the original monosaccharide into intermediate compounds which could not possibly occur in animal cells and by the use of reagents destructive to living matter. A partial explanation of these conversions, *in vivo*, has been obtained. It consists in the transformation of monosaccharides into glycogen, this process occurring chiefly in the liver.

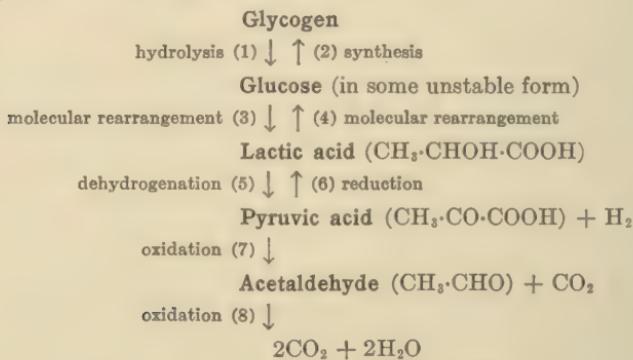
The famous discoveries of Claude Bernard showed that glycogen is formed in the liver when the animal receives carbohydrate food. Any excess of sugar in the portal blood is stored away as liver glycogen. As Claude Bernard also showed, glycogen is paid out from the liver into the blood in the form of glucose. The work of later investigators, chiefly Carl Voit, showed that all the carbohydrates, ordinarily eaten by a mammal, serve to increase the glycogen store of the liver. When the liver is perfused with blood containing any of the monosaccharides that can be derived from digestion, glycogen is increased in the liver. Glycogen is

also formed in other tissues. A little of it is found in mammalian muscle and much larger amounts in muscles of cold-blooded animals. It occurs in traces in most animal tissues. The wide distribution of glycogen leads to the tentative hypothesis that any carbohydrate may be converted to glycogen in the animal before it is actually utilized. This idea seems all the more probable in the light of Embden's work on the oxidation of carbohydrate in muscles. Inasmuch as glycogen is converted by hydrolysis into glucose, any carbohydrate that serves as a glycogen former might be converted into glucose through the stage of glycogen. This resolves the problem of sugar conversion in the animal organism into a question as to how cells convert sugar into glycogen. No definite answer to this question has been obtained as yet.

The Regulation of Blood Sugar.—The conversion of glycogen to glucose in the liver is known to occur in response to electrical stimulation of the splanchnic nerve. Such stimuli can probably occur under the control of the central nervous system. This appears to be the case because, as Claude Bernard showed, puncture of a certain spot in the floor of the fourth ventricle of the brain causes a distinct increase in blood sugar with a corresponding decrease of the glycogen store in the liver. This operation is called sugar puncture. It causes the blood sugar to become sufficiently concentrated to produce glycosuria. Moreover, the blood sugar rises under emotional stimuli, thus giving further evidence of nervous control of the glycogen breakdown in the liver. As will be explained later (p. 716) this is due, in part at least, to an indirect effect in which the nerve stimuli act upon the adrenal glands. This causes the secretion of adrenine into the blood. Adrenine excites the liver to transform glycogen into glucose. The liver contains an enzyme, **glycogenase**, which effects this hydrolysis. The control of the liberation of sugar from the liver is in some way adjusted to the needs of the body and this means, in the main, to the needs of the muscles for carbohydrate. The more rapidly sugar is used by the muscles, the more rapidly is it liberated from the liver. This adjustment, whether it is entirely controlled by the nervous system or whether it is due to minute changes in the composition of the blood is, in any case, sufficiently exact to secure great constancy of the sugar content of the blood. On a carbohydrate-free diet, an animal or a man shows a tendency to maintain constancy of the blood sugar even after all the glycogen store is exhausted. This is possible because of the ability of the animal body to transform glycerol, fatty acids and some of the acids derived from deamination of amino-acids, into glucose. This ability is not adequate to enable the human body to metabolize normally after its glycogen store is exhausted. The abnormalities that occur will be described presently. But in spite of this exception, the animal body shows a remarkable ability to maintain constancy of the quantity and the quality of its blood carbohydrate.

The Form of Glucose Used.—As explained in Chap. IV., glucose exists in no less than five different forms. Several of these doubtless exist in the blood. Possibly the simple form of glucose with four asymmetric carbon atoms is not the one used by the body. Only traces of it ever exist in any glucose solution. The γ form has been found to be produced when glucose comes in contact with the walls of the intestine. This form is more readily oxidized *in vitro* than are the simple and the lactone forms. This suggests the possibility that the γ form may be of special service in physiological oxidation. So far as oxidation in muscle is concerned, the hexose phosphate derived from glycogen is the readily usable form, according to Embden. It exists in the muscle in the form of larger complex called "lactacidogen."

Intermediate Products.—The series of linked reactions which together constitute the process of carbohydrate oxidation in the animal has been extensively investigated. Certain of the details are represented in the following scheme. The double arrows represent reversible reactions, the single arrows, those that do not occur reversibly. The different reactions are numbered for reference.



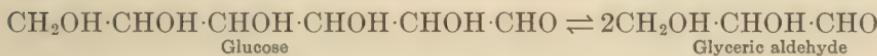
It is convenient to discuss these reactions separately.

1. The hydrolysis of glycogen differs from the well-known polysaccharide hydrolyses only in that typical glucose is probably not formed as a preliminary to oxidation although it is produced when it is to be put out of the cell into the blood. If it is to be oxidized it takes a special form, probably that occurring in lactacidogen.

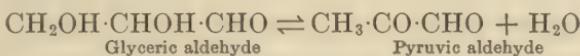
2. Synthesis of glycogen, like many biological syntheses, cannot be imitated as yet by artificial means; but the process has been amply demonstrated to occur in animal tissues. This reaction depends upon protoplasmic structure and does not occur in dead cells.

3. The change from glucose to lactic acid involves cleavage and a molecular rearrangement that need not necessarily have any relation to oxidation or reduction. This is seen from the fact that *two* molecules of lactic acid ($\text{C}_3\text{H}_6\text{O}_3$) have the same elementary composition as *one*

molecule of glucose ($C_6H_{12}O_6$). The reaction occurs not only in muscle, as was shown above, but also in liver. When liver is perfused with blood containing an excess of glucose or when a glycogen-rich liver is perfused with ordinary blood, lactic acid is given off. The nature of this molecular rearrangement has been investigated extensively. Compounds that are possible intermediates in artificial transformations of glucose to lactic acid have been administered to animals in various ways to test the production of either sugar or lactic acid. The compounds that have been tried are unstable aldehydes, such as **glyceric aldehyde**, also called **glycerose** ($CH_2OH \cdot CHOH \cdot CHO$) and **pyruvic aldehyde**, also called **methyl-glyoxal** ($CH_3 \cdot CO \cdot CHO$). Either of these can be converted into lactic acid by living organs or by macerated tissue preparations. Glyceric aldehyde could be produced, theoretically, by glucose splitting.



Pyruvic aldehyde could be produced, theoretically, by dehydration of glyceric aldehyde.



Dakin has shown that many tissues and especially liver and muscle contain an enzyme, **glyoxalase**, which readily converts pyruvic aldehyde and similar compounds to lactic acid. Neither glyceral nor pyruvic aldehyde has ever been isolated from tissues. If they are intermediates in the transformation of sugar to lactic acid, they must be very transitory. Their instability is in accord with the theory. Phosphates probably participate in this transformation as shown by Emden. The same is true in the case of lactic acid-producing fermentation in yeasts and bacteria, as shown by several investigators. Phosphates have also been shown to further the oxidation of glucose by hydrogen peroxide *in vitro*.

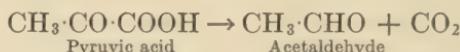
4. The production of sugar from lactic acid is known to occur in certain organs. Liver, perfused with blood containing lactic acid, increases its glycogen. The lactic acid formed during muscular contraction is more, according to satisfactory estimate, than that which is oxidized. Presumably, some of it is resynthesized to sugar. This process is doubtless a reversion of the reactions which produce lactic acid.

5. The dehydrogenation of lactic acid to pyruvic acid is difficult to demonstrate directly because pyruvic acid is readily oxidized. But traces of it have been obtained along with lactic acid when the amino-acid, alanine ($\text{CH}_3\text{CHNH}_2\text{COOH}$), is administered in excess to the body. Alanine is readily transformed into lactic acid which might well give rise to the pyruvic acid. Moreover, when oxidation in the body is so

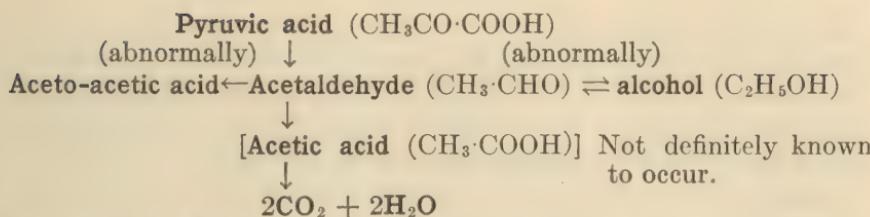
deranged that lactic acid fails to be oxidized but is changed to aceto-acetic acid, also called diacetic acid ($\text{CH}_3\cdot\text{CO}\cdot\text{CH}_2\cdot\text{COOH}$) pyruvic acid also gives rise to aceto-acetic acid. Pyruvic acid readily oxidizes in the normal body to $\text{CO}_2 + \text{H}_2\text{O}$. But the best evidence for the occurrence of pyruvic acid as an intermediate in lactic acid oxidation is, perhaps, the reverse reaction, the reduction of pyruvic to lactic acid.

6. Injected in excess, pyruvic acid gives rise to lactic acid and glucose in the urine. Perfused through the liver, pyruvic acid causes the formation of lactic acid. Alanine can also be produced in the body from pyruvic acid as well as from lactic acid. Dakin has obtained evidence to show that the reduction of pyruvic acid to lactic acid does not occur unless conditions are such as to depress oxidation. This clearly indicates that normal oxidation produces the reverse reaction: lactic acid \rightarrow pyruvic acid + H_2 . The presence of a hydrogen acceptor in active cells is in agreement with this idea.

7. Oxidation of pyruvic acid to acetaldehyde and CO_2 has not been completely demonstrated but probably occurs as is shown by several lines of indirect evidence. Acetaldehyde has not been actually recognized in animal tissues but this does not disprove its formation because it might be so rapidly oxidized or otherwise changed as to escape detection. Pyruvic acid, added to the juice pressed out of yeast is fermented to acetaldehyde and CO_2 .



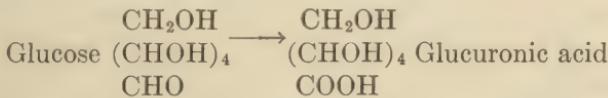
Acetaldehyde is very probably an intermediate in yeast alcoholic fermentation and this has a bearing upon animal metabolism because Emden has shown that when blood containing acetaldehyde is perfused through the liver, alcohol is formed. The reverse reaction is a possibility in that liver perfused with blood containing alcohol forms some kind of aldehyde. This raises the question as to whether alcohol normally occurs in animal metabolism. Alcohol has been identified in distillates from fresh muscle and other tissues. Its presence seems to be associated with conditions of defective oxidation. This suggests that alcohol can be produced in the course of animal oxidations, though it is not, normally. But inasmuch as the reaction, acetaldehyde \rightleftharpoons alcohol, is known to be reversible, even the abnormal occurrence of alcohol in animal tissues suggests that acetaldehyde is produced in them. Another line of evidence is found in the production of aceto-acetic acid. As explained above, this substance is formed from lactic or pyruvic acid and therefore from glucose when oxidation is defective. The change of lactic acid or pyruvic acid to aceto-acetic acid can be effected, *in vitro*, through the intermediate stage of acetaldehyde. The theory of the normal and abnormal processes involving acetaldehyde is represented schematically thus:



The reactions: Pyruvic acid → acetaldehyde → acetic acid → CO₂ + H₂O, represent the theory of the *normal* course of oxidation. The reactions, producing aceto-acetic acid and alcohol, represent the disposal of a part of the carbohydrate during *abnormal* (defective) oxidation.

8. The oxidation of acetaldehyde to CO₂ + H₂O is usually represented as going through acetic acid. This idea is based chiefly on the fact that abnormally large amounts of pyruvic acid, when introduced into the body, give rise to acetic acid.

Direct Oxidation of Glucose to Glucuronic Acid.—Under some conditions, glucose itself is oxidized. When camphor, menthol, naphthol or certain other aromatic compounds or when certain drugs, such as morphine or chloral hydrate are fed, they are partly excreted in the urine as **glucuronates**, that is, as esters of glucuronic acid. When the body is largely depleted of its carbohydrate by starvation, camphor causes the excretion of only a trace of glucuronic acid; but if camphor and sugar are then given together, large amounts of glucuronic acid appear. This indicates that glucose is oxidized to glucuronic acid. The reaction readily occurs *in vitro*. It may be represented thus:



This reaction is not the normal mode of oxidation of more than a minute fraction of the body carbohydrate. This is shown by the fact that glucuronic acid, if fed or injected, is excreted in the urine almost quantitatively. Glucuronates are found normally in human urine. They are chiefly compounds of glucuronic acid with phenol derivatives which probably arise from putrefaction in the intestine. But the amount of glucuronates is normally very small, only a few miligrams per day. In diabetes, however, larger amounts are excreted. This gives further evidence that direct oxidation to glucuronic acid is the abnormal rather than the normal fate of glucose in animals.

The Oxidation of Fats.—The origin of animal fat from the foods was discussed above (p. 66). The storage of fats corresponds to that of glycogen. Several different tissues are important storage places. The liver, the bone marrow and in the salmon even the muscles serve this purpose. But adipose tissue contains by far the largest store of fat in

most animals. The relation of the liver to fat metabolism is somewhat complex. In this organ, fats are not merely stored but appear to be chemically altered. As is well known, liver fats are always oily even though fat from other parts of the animal may be comparatively hard. This is an index of desaturation of fatty acids in the liver. Unsaturated fatty acids, such as occur in oily fats, are more readily oxidized than are the saturated ones. Some of them, as explained above, are even autoxidizable. The liver is rich in lecithin and this fact may be related to the process of desaturation which appears to occur in the liver, for lecithins always contain unsaturated fatty acid.

The transport of fat does not normally occur in the blood in the form of actual free neutral fat to any important extent. The researches of Bloor have made it highly probable that fat is transported largely in the form of lecithin. This is doubtless an advantage in that lecithin mixes with water and water solutions such as blood plasma to form a finely dispersed colloidal solution. Fat itself can be only emulsified in blood plasma and such emulsions cannot pass through the membranes of most cells as can lecithin.

The regulation of the fat and lipoid content of the blood is almost as exact as is that of the sugar content. The mechanism of this regulation has not been extensively investigated.

The utilization of fats, that is, their actual oxidation, may begin with lecithin. Meigs has shown, by analyses to determine phospholipins in the arterial and venous blood of the mammary gland, that the amount of such substances removed by the gland is sufficient to account for all of the milk fats secreted. This shows that lecithin is taken into a gland for purposes of secretion. That it is similarly taken up when it is to be oxidized remains to be proved. The tendency of the body to maintain a constant proportion of lecithin seems significant. The older view that fat is oxidized as such has not received support from newer investigations. The paucity of lipase in many tissues that metabolize fat has been emphasized. If fat were used as such, a lipase would probably be required for its initial hydrolysis. On the other hand, tissues are well supplied with enzymes, the so-called **esterases**, which can split lecithins. The probability that phosphates participate in the initial stages of sugar utilization adds interest to the hypothesis, advanced by Bloor, namely, that phosphorized fats (lecithins) are concerned with fat utilization and are perhaps the initial stage of fat oxidation.

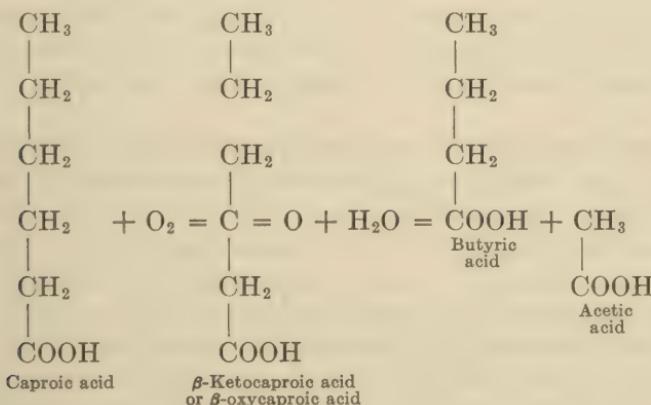
The Theory of β Oxidation of Fatty Acids.—Both the glycerol and the fatty acid parts of the fat molecule are subject to physiological oxidation but not necessarily by means of the same reactions. It is convenient to discuss the oxidation of glycerol and fatty acid separately.

Knoop proposed a theory of the oxidation of fatty acids. He found that, in animals, fatty acids tend to give rise to hydroxy-acids in which

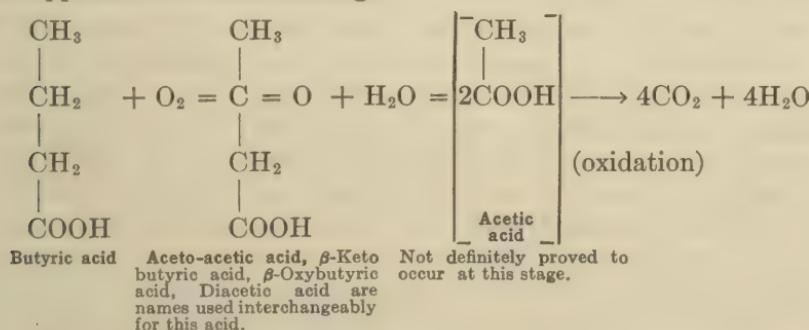
the —OH group is in the β position. According to this theory, oxidation of all the higher fatty acids involves an initial attack of oxygen upon the hydrogen attached to the β carbon atom. The reactions representing this process were shown in Chap. III (p. 59). Confirmatory evidence for this theory has been obtained by numerous investigators. The work of Dakin has been especially significant. He has shown that the oxidation of fatty acids by H_2O_2 *in vitro* also follows the course of β oxidation. The first step in the oxidation *in vitro* and, presumably, *in vivo*, is the production of a β ketone acid, that is, the fatty acid is transformed into

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an acid with a ketone group ($\text{C} = \text{O}$), in the β position. *By reduction,*

|
taking on two hydrogen atoms, the ketone acid may change to the corresponding hydroxy-acid. Because of these reactions, hydroxy-acids are of the β type when produced in the body. But *by oxidation*, the ketone acid is broken down to yield acids of smaller molecular weight. The reactions by which caproic acid undergoes partial oxidation may be represented thus:



Later steps in the oxidation, leading to the production of $\text{CO}_2 + \text{H}_2\text{O}$, are supposed to be the following:



Large amounts of aceto-acetic and its derivatives are formed in the body during conditions of defective oxidation such as occurs in diabetes. This lends weight to the idea that this acid is produced normally in the course of metabolism, but is oxidized to $\text{CO}_2 + \text{H}_2\text{O}$. The production of aceto-acetic acid in the normal course of oxidation is indicated by the fact that traces of it are usually present in human urine.

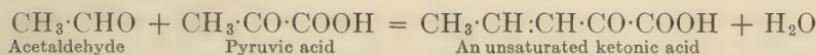
The oxidation of propionic and other acids, having an odd number of carbon atoms, probably follows a different course. Propionic acid is derived from the deamination of several amino-acids. It is not derived from the fats. It can be changed in the body to lactic acid and it is then oxidized through pyruvic acid in the usual way. As an approximate generalization it is assumed that acids containing an even number of carbon atoms are oxidized through the stage of aceto-acetic acid, while acids with an odd number of carbon atoms are oxidized through the stage of lactic acid.

Oxidation of Glycerol.—Glycerol is known to be convertible in the body into lactic acid. The liver, perfused with blood containing glycerol, gives off lactic acid. This process is reversible in that lactic acid can give rise to glycerol in the liver. Other confirmatory evidence of the interconvertibility of glycerol and lactic acid has been obtained; so that it is now generally conceded that the oxidation of glycerol pursues the same course as that of glucose, passing through the lactic acid and pyruvic acid stages.

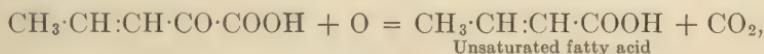
The Interrelation between the Oxidations of Sugar, Amino-acids and Fats.—During the utilization of glucose and glycerol and of the propionic acid, derived from several of the amino-acids, all three of these substances are converted or, at least, are convertible into lactic acid. Proceeding from the lactic acid stage, utilization of these metabolites involves processes of oxidation that are apparently identical in each case. But all the reactions for production of lactic acid in the body are reversible and even the first stage in its oxidation, lactic acid \rightleftharpoons pyruvic acid, is also reversible. If thus comes about that the animal can resynthesize glucose, glycerol and certain amino-acids by using lactic or pyruvic acid as a starting point. These processes can occur irrespective of the source from which the acid was produced. This furnishes an explanation of some of the chemical processes by which the animal effects interconversions of carbohydrates, fats and amino-acids. It does not explain the production of the fatty acids of fats. These are all of the type characterized by an even number of carbon atoms in their molecules. But lactic acid is formed by the oxidation of acids with an odd number of carbon atoms and presumably the reverse reactions, reduction and synthesis, would produce fatty acids of this type.

The synthesis of typical fatty acids with an even number of carbon atoms might occur, theoretically, by means of a synthetic union of

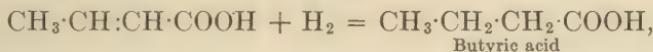
pyruvic acid or other ketonic acid with acetaldehyde. Both of these substances appear to be produced from lactic acid in the body. Their union could occur thus:



Similar unions between aldehydes and ketonic acids have been made *in vitro*. The unsaturated ketonic acid, so produced, can be oxidized. For example, the reaction,



would produce an unsaturated fatty acid. If this were then subjected to reduction (hydrogenation), a normal, saturated fatty acid would be formed. For example, the reaction,



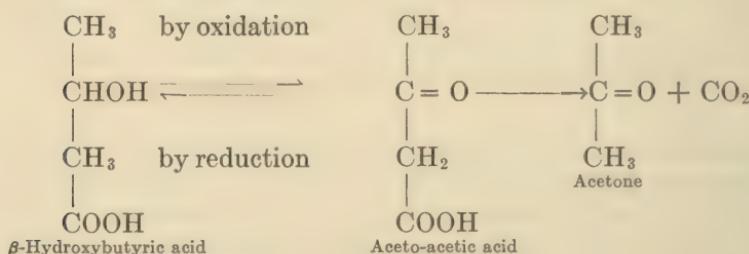
would produce butyric acid if the original starting point of the process were pyruvic acid and acetaldehyde. In this way, a fatty acid with an even number of carbon atoms is producible from a ketonic acid having an odd number of carbon atoms. Reactions, comparable to all of these, have been obtained *in vitro*. Inasmuch as numerous different ketonic acids and aldehydes can serve as the starting point, the process appears to be a general one. It thus affords a theoretical explanation of the synthesis of fatty acids with long carbon chains. They would appear to be built up by the successive syntheses of acetaldehyde with higher and higher ketonic acids, each of which can be produced from the corresponding fatty acid. But whatever may be the process of fatty acid synthesis *in vivo*, fats are certainly formed in animals from carbohydrates and to a limited extent from proteins.

The failure of the animal body to synthesize any but the simpler amino-acids and the consequent inability of the animal to synthesize protein, when certain amino-acids are not supplied, was discussed above. It is clear, then, that the interconvertibility of proteins, fats and carbohydrates, though possible in animals, has certain marked limitations particularly as regards the production of proteins. Plants are less limited in this respect, but their intermediary metabolism has not been studied extensively.

The metabolism of proteins, fats and carbohydrates shows further interrelation: (1) The dependence of the process of fatty acid oxidation upon sugar oxidation and (2) the conversion of fats and proteins to glucose when carbohydrate is not oxidized in adequate amounts. This inadequacy occurs in man during starvation, during the use of a diet deficient or lacking in carbohydrate and during diabetes. Diabetes will be described later in connection with its cause, a failure of the internal secretion of the pancreas. It is sufficient for the present to state that any

condition in which carbohydrate is not oxidized, as is the case in diabetes, causes abnormality in fatty acid oxidation and increased production of sugar from fat and protein.

The abnormality in oxidation is readily detected by the excretion of acetone in the breath and of this and other partial oxidation products of the fatty acids in the urine. Fatty acids apparently oxidize as far as the aceto-acetic acid stage, but from here on, oxidation fails. Instead of being completely oxidized, this acid is partly excreted and is partly changed to β -hydroxybutyric acid and acetone. The interrelations of these three compounds are represented by the reactions which follow:



Any or all of these three compounds appear in the urine; but as acetone is comparatively easy of detection and is always excreted during defective animal oxidation, the presence of these substances in urine is commonly called **acetonuria**. Only faint traces of these compounds, amounts so small as to escape detection by ordinary tests, appear in normal human urine. The β -hydroxybutyric and aceto-acetic acids tend to break down the regulation of the hydrogen ion concentration of the blood or at least to decrease its alkali reserve (CO_2 transporting power). On this account, the body is said to be in a condition of **acidosis** even though the blood is not acid and its pH is but slightly altered from the normal. The depletion of the CO_2 transporting power of the blood and other effects accompanying acidosis cause a still further depression of oxidative processes in the cells. Thus a vicious circle arises and leads to the immediate cause of death from diabetes.

The abnormality in sugar production occurs in advanced stages of diabetes. Even on a carbohydrate-free diet, large amounts of sugar appear in the urine. This is true even when the subject is emaciated and fats are not abundantly eaten. Sugar in this condition can come from proteins as well as from fats. It is as if the body, though unable to use sugar, demands more and more of it. The extent, to which this abnormal sugar production may go, has been investigated in human diabetics and in animals rendered diabetic by removal of the pancreas. Such observations show that fats are converted almost quantitatively to glucose and that certain amino-acids are also converted into glucose in amounts that

can be foretold from the structure of the amino-acid. For example, amino-acids which break down to produce propionic acid yield glucose in amounts equivalent to one molecule of glucose for two molecules of propionic acid. The amino-acid, glycine ($\text{CH}_2\text{HN}_2\cdot\text{COOH}$) is also converted almost quantitatively to glucose in the completely diabetic animal. Lusk, who investigated this conversion, calculates that one molecule of glucose is excreted in the urine of diabetic animals for every three molecules of glycine fed to them. Inasmuch as glycine is a derivative of acetic acid rather than of propionic acid, this glucose formation requires a special explanation to account for the intermediate reactions. This explanation has not yet been obtained.

The facts of deranged metabolism show that the processes involved in utilization of glucose, fats and amino-acids do not proceed independently. Even the entire series of complex reactions which constitute the breakdown and final oxidation of any one of these metabolites is not an independent process. All oxidations are linked in some way by the mutual effects of concurrent reactions upon one another. This generalization can be carried still further. The oxidations in any one organ are not entirely independent of metabolic processes in other parts of the body. The rate and the character of the oxidation in the muscles, for example, determine, in some measure, the cause of metabolic changes in the liver and elsewhere. Conversely, the chemical changes occurring in the liver, changes such as the liberation of sugar and of fat into the blood, exert certain limitations upon muscle metabolism. This correlation is partly determined by the integrating action of the nervous system which controls excitation of skeletal muscles and at the same time, controls, to some extent, the metabolism of the liver and other organs. But this correlation is also effected, in part, by the chemical composition of the blood. As in respiration, so in oxidation, the organism behaves as a unit and not as an aggregation of separate organs.

The Balanced Relation between the Oxidation of Glucose and that of Fatty Acid.—The interdependence of metabolic reactions is further shown in the quantitative relationships which must be maintained between the sugar and the fatty acids that are undergoing oxidation in order that oxidation shall be normal. Abnormality is indicated by the failure to oxidize aceto-acetic acid. Fatty acids which produce aceto-acetic acid in animals are called **ketogenic**. Glucose and substances, which oxidize like glucose in such a way as to prevent the appearance of aceto-acetic acid and its derivatives are called **antiketogenic**. Schaffer has shown that if a given amount of ketogenic substance is being oxidized, a certain minimal amount of antiketogenic substances must be simultaneously oxidized in order to prevent acetonuria. He first showed that the oxidation of aceto-acetic acid, *in vitro*, in an alkaline solution, is very slow without glucose, but occurs readily with glucose. In a sufficiently alkaline

solution, it even occurs at body temperature. Glycerol can replace glucose in this reaction, but lactic acid cannot. If a glucose solution is first made alkaline and then used for promoting the oxidation of aceto-acetic acid, it acts much more rapidly, at a given temperature and alkalinity, than does freshly dissolved glucose. These facts led Schaffer to suggest that an intermediate product of the breakdown of glucose forms a compound with aceto-acetic and that, in this form, the simultaneous oxidation of glucose and aceto-acetic acid begins.

The quantitative relations between ketogenic and anti ketogenic substances, required for normal metabolism, have been studied by Schaffer and other investigators. The outcome of such studies may be summarized in the statement that one molecule of glucose is required to secure the oxidation of each molecule of aceto-acetic that is produced by the partial oxidation of ketogenic substances. To show this, the amount of glucose which can be produced in the body from glycerol and amino-acids, must be calculated as well as the glucose which can be produced from the carbohydrates of the food. Such calculations must be based on certain assumptions with regard to the probable course which amino-acids, fats and carbohydrates would take in metabolism. But in spite of these approximations, the theoretical figures, deduced from the calculations, compare very well with results actually obtained in dietary tests. It is shown, for example, by calculation, that a diet containing protein to the extent of 10 per cent of its fuel value (calories), fat to the extent of 80 per cent and carbohydrate to the extent of 10 per cent of the calorie value would yield one molecule of glucose to one of aceto-acetic acid produced by partial oxidation of the fatty acids. This diet, then, should be just on the borderline between diets that produce acetonuria and those that do not. Practical tests show this to be the case. It does not produce acetonuria in a normal human being even when the subject of the experiment has been rendered "glycogen-free" by a previous short period of starvation. But any increase of fat with a corresponding decrease of carbohydrate in the diet does tend to produce acetonuria; while the reverse change, a proportional increase of carbohydrate with a corresponding decrease in fat of the diet does not produce acetonuria. In other words, the proportion of glucose (glucose from carbohydrates + glucose from other sources) to fatty acids cannot be reduced below a certain minimum without causing failure of the complete oxidation of fatty acids. This failure is regarded by Schaffer as a disturbance in the "ketogenic-antiketogenic balance." This balance can be maintained only when glucose is available for oxidation in sufficient amounts. This explains why diabetic patients have marked acetonuria with accompanying acidosis; for in them, glucose, though present in the body, is not available for use by the defective oxidizing mechanisms of their cells. It also explains and establishes what was earlier suspected, namely, the

futility of attempting to cure a diabetic patient by the use of a carbohydrate-free and fat-rich diet.

The Respiratory Quotient.—The ratio of carbon dioxide produced to oxygen utilized by an animal is the respiratory quotient. It is measured in volumes, that is, the volume of the CO₂ given off is divided by the volume of the O₂ absorbed. Inasmuch as equal volumes of all gases contain the same number of molecules at the same temperature and pressure, volume measurements of gases are convenient for chemical calculations. The respiratory quotient has been used extensively in the study of physiological oxidations because it furnishes an index of the nature of the metabolites that are oxidized. Certain precautions must be observed in taking respiratory quotients in order that the results shall be capable of a correct interpretation. The chief of these precautions are the ones which insure that the carbon dioxide measured shall be representative of that which is produced during the time that oxygen utilization is measured. In observations on man, this requires uniformity of lung ventilation throughout the period of observation. In some cases, especially in experiments upon animals, it is necessary to maintain observations of the respiratory exchange over a long period in order to avoid the effect of inequalities in the rate of CO₂ elimination. The respiratory quotient usually lies between 0.75 and 0.89 for human beings. For herbivores, it is higher; for carnivores, lower. Variations depend upon the composition of the metabolites that are being oxidized. This fact is shown in a general way, by rough calculations of the oxygen used and the carbon dioxide produced during complete oxidation of each of the types of foods. For example, the oxidation of glucose can be represented empirically as:



This shows that for the complete oxidation of carbohydrate, the volume of O₂ used is equal to that of CO₂ produced, so that the respiratory quotient is unity (R.Q. = 1). Similarly, the complete oxidation of triolein, as an example of a fat, may be represented, empirically, by the equation:



In this case, the respiratory quotient is $\frac{54}{77}$ (R.Q. = 0.70). For proteins, the calculation is more complex. This is the case because: Their composition is more variable; the amount used for tissue repair and the mode of its use is uncertain; and a large part of the amino-acids, derived from protein, is transformed into waste products without being completely oxidized. A determination of the nitrogen compounds of the urine, however, makes possible a calculation of the amount of protein material that is being metabolized and the proportion of it that is not completely oxidized. Using these calculations and assuming that a certain average

combination of amino-acids is being metabolized, one can estimate the respiratory quotient for protein oxidation. The average of such estimates is commonly given as R.Q. = 0.80. Any R.Q. higher than 0.80 indicates the oxidation of carbohydrate in excess of protein and fat, while a quotient less than 0.80 indicates that fat oxidation predominates. The larger the amount of carbohydrate metabolized, in proportion to that of fats and proteins, the nearer the respiratory quotient approaches to unity; the larger the amount of fat metabolized in proportion to the other foods, the nearer the respiratory quotient approaches to 0.70. For example, a man, doing muscular work on a diet composed almost exclusively of carbohydrate, has a respiratory quotient of 0.9 or more; but if his diet is predominatingly fatty foods, the respiratory quotient may be as low as 0.73. Calculations, based on theoretical formulas, permit estimations of the proportional amounts of proteins, fats and carbohydrates used during the time that a given respiratory quotient prevails. Respiratory quotients, larger than unity, have been observed. They are explained, in some cases, by the fact that CO_2 is given off during the observation more rapidly than it is produced. This condition can arise through an increase in the rate of lung ventilation or increased blood flow or a combination of these causes. The respiratory quotient may be increased by the formation of fat from carbohydrate. In this process, the oxygen-poor fats are synthesized from the comparatively oxygen-rich glucose. This is accompanied by the liberation of CO_2 . Respiratory quotients in excess of unity have been claimed to be due to rapid fat synthesis during excessive carbohydrate feeding. During the rapid fattening of a goose, for example, respiratory quotients as high as 1.50 have been obtained by measurements that seem trustworthy. On the other hand, the formation of carbohydrate from fat might result, theoretically, in a respiratory quotient less than 0.70. Quotients as low as 0.53 have been observed in hibernating animals and have been regarded as evidence for the conversion of fat to storage carbohydrate. Glycogen, however, has not been shown to increase in the hibernating animal. Yet glycogen would have to be formed in amounts far in excess of those ever found in animals in order to produce these low respiratory quotients. Carbohydrate storage, at most, can account for only a small part of the high proportion of O_2 used to CO_2 produced during hibernation. Other factors, especially defective oxidation, may explain these low quotients. This is indicated by the fact that lactic acid is found in notable quantity in the urine during hibernation.

Heat Production and Regulation.—The maintenance of a constant temperature, such as prevails in warm-blooded animals, requires a nice adjustment between heat production and heat loss. The advantage which the organism attains from constancy of body temperature is a regulation of the rate of chemical reactions. Many interrelated reactions

must go on at rates that have a certain relation to each other. These reactions are, in many cases, differently affected by temperature changes. On this account, balanced relations among the many metabolic reactions of the body can be maintained better at constant temperature than during temperature fluctuations.

Temperature regulation is secured by two types of responses to temperature changes in the environment, namely, changes in heat production and changes in the rate of heat loss. Changes in heat production are determined chiefly by the relative amount of muscular contraction, though other organs are also concerned. But in any case, the rate of oxidation determines heat production. An increase of oxidation can compensate for falling temperature and a decrease for rising ones. Changes in the rate of heat loss are determined: (1) Partly by the extent of lung ventilation, because air is warmed as it is expired; (2) partly by the relative amount of evaporation. Because of the high latent heat of water much heat is used in evaporating it from the sweat on the skin, from secretions on the mucous linings of the respiratory passages, the mouth, etc. (3) The rate of heat radiation from the body surfaces is also a factor. On the average, over 70 per cent of the heat loss of the human body occurs in this way. The amount of heat brought to the skin is subject to regulation by means of vaso-motor control of the blood flow and by changes in the volume of the blood.

Primitive Heat Regulation.—Cold-blooded (poikilothermic) animals are not all entirely devoid of some temperature regulation, although in molluscs and some other aquatic forms that have been investigated temperature regulation cannot be detected. In these forms, and doubtless in all the lower invertebrates, the body temperature is always that of the environment. The rate of oxidation, as measured by O_2 intake and CO_2 output is proportional to the temperature. The graph relating temperature to rate of oxidation is practically a straight line within the temperature limits that are compatible with the normal life of the organism. But in some of the invertebrates this is not the case. In earthworms, for example, Vernon showed that warming from 10 to 22.5°C. caused no increase in their CO_2 output. Inasmuch as oxidations *in vitro* are more than doubled in rate by such a rise in temperature, these experiments show that even these lowly forms must have some mechanism for restraining oxidation. They appear to have no means for the regulation of heat loss. Lizards have a relatively constant CO_2 production between 10 and 35°C. but above or below these temperatures, their rate of oxidation is parallel to temperature changes.

The Echidna (the Australian ant-eater) may be classed as the lowest of the warm-blooded animals. Its temperature regulation is primitive. Although its temperature varies with that of the environment, it changes only 10°C. when the air is changed from 5 to 35°C. This animal

hibernates in cold weather and during hibernation, it maintains its body temperature only slightly higher ($0.5^{\circ}\text{C}.$) than that of the air. Temperature regulation is thus shown to be abolished by hibernation. This is in agreement with the fact that the body temperature of the hedgehog and of other hibernating mammals is markedly decreased during hibernation. Temperature regulation in the Echidna appears to be effected by changes in the rate of oxidation. The animal has no sweat glands and does not make vasomotor adjustments to temperature.

The general conclusion from these observations is, then, that temperature regulation by control of oxidative heat production is the primitive type. It probably marks the beginning of the evolution of heat regulating mechanisms. Marsupials are transitional in respect to heat regulation. They not only regulate heat production but also regulate, to some extent, the loss of heat.

Heat Regulation in Higher Mammals.—In man and in all typical mammals, temperature regulation at certain environmental temperatures (from about $15^{\circ}\text{C}.$ to about $37^{\circ}\text{C}.$) is effected almost entirely by control of the rate of heat loss. Below $15^{\circ}\text{C}.$ shivering occurs. This is an involuntary mechanism for increase of heat production. It may be augmented by voluntary muscular activity, but in any case, oxidation is speeded up in the muscles. With a rise of temperature, decreased muscular activity, and therefore decreased oxidative heat production, may occur to some extent; but if the animal is quiet anyway, it cannot compensate for rising temperature by any further diminution of oxidation. Indeed, oxidation is commonly increased. This necessitates temperature control by regulation of heat loss. The factors involved in this regulation are of varying effectiveness.

Numerous researches, especially those of Barbour and his co-workers, have shown that a fundamental factor is the change in the water content of the blood. With falling environmental temperatures, the blood becomes more concentrated, with rising temperatures, more dilute. Water is withdrawn from the blood to the tissues in the former case and moves into the blood from the tissues in the latter. This movement is reflected in changes in the blood viscosity. The osmotic pressure of the blood does not greatly change because salts (diffusible constituents) move in and out of the blood stream with the water, but the colloids of the blood are not diffusible and remain in the blood. It thus results that the solids of the blood increase in proportion to the water during cooling and decrease during warming. The total blood volume varies accordingly. By this means, water, the chief heat transporter of the body, is kept in the tissues in increasing amounts during exposure to cold, but is freed to convey heat to the radiating surfaces of the body during exposure to heat. Concentration of the blood by injection of excessive amounts of glucose or salt causes a marked rise of body temperature.

With increase in blood volume, there may be a marked increase in secretion of sweat. In man, evaporation of sweat from the skin constitutes one of the most important cooling factors. In other mammals, which possess few or no sweat glands, this factor is of slight or no aid. In dogs, evaporation of freely secreted saliva from the mouth and the hanging-out tongue constitutes an important cooling device. In horses, both sweat and salivary secretions aid in cooling.

Increased lung ventilation, with rising temperature, is another factor which is important in some species. The panting of a dog in hot weather is very noticeable. This adjustment is called *heat polypnea*. It may occur even when it is not recognizable to casual observation of the respiratory rate. Increase in lung ventilation, effected by deeper breathing as well as by faster breathing, can increase the heat loss, especially when the air has low humidity which favors evaporation.

Another important factor in the regulation of heat loss is the control of the relative amount of blood flow through the surface regions. Obviously, any increase in the amount of blood flowing through the skin and exposed mucous membranes favors transport of heat from the deeper regions of the body to these radiating surfaces. Conversely, decreased blood supplies to the surfaces favor the conservation of body heat. This temperature regulating factor works in conjunction with the changes in the water content of the blood.

Nervous Stimuli Concerned with Heat Regulation.—The motor nerves to the skeletal muscles convey stimuli which cause muscular excitation and thus indirectly determine the amount of the major part of oxidative metabolism in muscles. These stimuli do not exclusively control muscular metabolism. Some oxidation occurs in a resting muscle. The sensory nerves, which reflexly excite shivering, are those connected with the cold receptors of the skin. This fact explains certain observations that would otherwise be very difficult to understand. It shows why shivering occurs during fevers when the body temperature is above normal. The feverish condition is accompanied by vasoconstrictor disturbances which decrease the blood supply to certain skin regions. This permits cooling of the skin and excitation of the cold receptors, so that shivering is reflexly produced. It is accompanied by a sensation of cold.

Secretory nerves to the sweat glands regulate their activity in connection with temperature control. The sensory nerves for this reflex are also those of the temperature receptors. Those for heat and cold are both involved. It has been shown, for example, that a man immersed up to the neck in a bath at 40°C. begins sweating upon the forehead without any change in the rectal temperature, but if, while still in the bath, one hand is immersed in cold water, the sweating immediately stops.

The vasoconstrictor nerves, by controlling the size of arteries and capillaries, are obviously concerned in temperature regulation. This means

that the vasomotor center and all the very numerous afferent influences which play upon it are also involved.

The motor nerves controlling respiratory muscles, the respiratory center and the afferent nerves which play upon it, must be involved, in some way, in the regulation of temperature.

Some nervous mechanism must be effective in the regulation of blood volume and concentration. Barbour has shown that after cutting of the spinal cord of the dog at the sixth cervical segment, the concentration of its blood remains constant when the animal is placed in a bath at 20°C., whereas, the same treatment of a normal dog causes a marked increase in the blood solids. Corresponding to these differences, the temperature of the operated dog falls rapidly but does not change in the normal control animal. The nerves concerned in the regulation of blood concentration have not been identified, but those which regulate the behavior of the walls of the capillaries might well be effective.

Heat Regulating Centers.—The mammalian body can be compared to a thermostat but it is much more complex than any artificial device for automatic temperature regulation. In the body, many mechanisms are involved. Each has its nervous control. Temperature regulation occurs only in animals with a complex centralized nervous system. This suggests that all the different nerve centers that are concerned with heat regulation, the skeletal-motor, vasomotor, reflex secretory, respiratory, etc., are subject to some coordinating device by which they are all made to act in concert for purposes of temperature regulation. The so-called heat puncture experiment gives an indication of the existence of such a device. This experiment consists in injuring a certain point in the thalamus at the base of the brain. It causes a fever which is a sign of the breakdown of temperature regulation. This does not necessarily prove the existence of a "heat center" because the result might be due to irritation of the brain rather than to the mere absence of a functional regulating center. The location, at which a puncture produces fever is, however, a fairly restricted one. Injuries to other parts of the brain do not cause comparable results. Moreover, the temperature of the blood entering the brain can largely control heat regulation. When the carotid blood is warmed, the animal grows colder as shown by a fall in rectal temperature. Cooling the carotid blood produces a rise in rectal temperature. Similar evidence is found in observations on shivering. An animal shivers when the temperature of the carotid blood is below 32°C. even though the rectal temperature is much higher. Conversely, the animal stops shivering when the carotid blood is at 32°C. even though the rectal temperature may be below 30°C. But the best evidence for the existence of a heat regulating center has been obtained in the experiments of Barbour and his co-workers. In this work, a technique was developed for inserting the closed end of a fine tube into a certain point at the base of the brain. By

running water of a given temperature through the tube, this locality of the brain could be warmed or cooled as desired. Although fever follows the operation, it soon subsides; but if water below body temperature is circulated through the tube, the rectal temperature of the animal rises. This result can be obtained only when the correct location in the brain is affected. Warming this spot causes the rectal temperature to fall. This center acts upon the production of heat. This fact has been demonstrated by measurements of the CO₂ output during changes in body temperature that result from warming and cooling of the center. For this reason, it is sometimes called a thermogenic center. But it is also concerned with the regulation of heat loss. Warming the carotid blood or the heat center causes vasodilatation in the skin region. This has been shown with dogs and rabbits. It is most easily observed in rabbits. In them the blood vessels of the ear are seen to dilate during warming of the center and to constrict during its cooling. Sweat secretion is also affected. This has been shown in experiments on dogs. Warming of the heat center causes secretion of sweat in their foot pads. The respiratory center is also subject to some influences from the heat center. Heat polypnea cannot be obtained in an animal which has been operated on to sever the connection between the respiratory center and the location of the heat center. This operation does not interfere with the regulation of respiration by effects other than those of temperature. It seems, then, that the heat regulating center exerts some control over the rate of heat loss as well as over the rate of oxidative heat production. Although these experiments depend upon direct excitation of the heat regulating center by thermal, electrical or mechanical stimuli, the failure of the body to successfully regulate temperature after destruction of the center suggests that it is a true reflex center through which afferent stimuli play upon the varied mechanisms that take part in temperature regulation.

Normal Temperature Variations.—Although the normal temperature is nearly constant, it shows slight variations within narrow limits. It varies somewhat with age, being higher in young animals than in adults. In human beings, fairly regular diurnal variations are also observed. Body temperature is lowest in early morning and highest in late afternoon or evening. This change is due chiefly to eating and to muscular exercise. Emotional conditions also exert an effect, partly upon heat production and partly upon heat loss. The diurnal variations are usually reversed in persons who sleep during the day and work at night. In travelling around the world, the temperature changes become adjusted to daylight conditions. Altogether, these normal variations seldom exceed 1.5°C. in human beings. The body temperature is usually between 37 and 38°C. The variations merely represent the lag of heat loss behind heat production together with changes in oxidative metabolism.

Fever.—Fever may be defined as any abnormally high temperature that is not due to food, exercise or environmental temperatures. It is usually due to a breakdown of the functioning of the heat regulating mechanism. Failure of the vasomotor adjustments and the regulation of blood concentration have been shown, in many cases at least, to precede increase in oxidative metabolism. The latter may not be increased at all. The total CO₂ production may be even below normal during fever. It has also been shown that after severing connections of the heat regulating center, certain infections which ordinarily produce fever do not have this effect. Drugs and toxins which produce fever and others which counteract it, appear to be able, in some cases, to act upon the heat regulating center, in that very low concentrations of these substances, when applied directly to the center, produce characteristic effects. One of the chief of the immediate effects of fever-producing conditions is an increased concentration of the blood. It has not yet been conclusively shown that this effect is primarily due to a failure of the heat regulating mechanism, although some evidence points to this explanation. In any case, the rise of temperature is largely, if not wholly, due to effects upon the nervous system. This idea is sometimes called the neurogenic theory of fever.

Relation between Oxidative Metabolism and Protoplasmic Structure.

Physiological oxidations are dependent upon the physico-chemical structure of protoplasm. Although the maintenance of structural integrity is an important factor in determining the normal course of all metabolism, its importance for physiological oxidation can be demonstrated in an especially striking way. Destruction of cells by shaking, by exposure to distilled water or by any other means, stops oxidative metabolism. Hydrolysis may go on in macerated cells and is even facilitated in some cases by cell destruction. The enzymes that are concerned with hydrolysis and some of those that catalyze certain oxidations (xanthine oxidase, uricase, tyrosinase, luciferase, etc.) continue to be active but typical oxidation of most metabolites stops with cell destruction and is greatly modified by minute changes in structure.

On the other hand, cell structure is itself dependent upon oxidation. Cytolysis sets in very rapidly, in the case of many cells, when oxygen is withdrawn. Some of the energy liberated by oxidation is required to maintain structure. This is shown by Meyerhof's studies of what is called the caloric quotient. This figure is obtained by dividing the heat evolved (expressed in calories) by the oxygen used (expressed in milligrams) during a given period. The caloric quotient has been determined for various cell foods when burned *in vitro* and is found to vary between 3.2 and 3.5. But if cells, such as invertebrate eggs which utilize no energy for contractile activity, are subjected to measurements of their heat production and their oxygen utilization, the caloric quotient is less

than 3. Even when every allowance is made for non-oxidative reactions, which might liberate heat in the cell, the caloric quotient is less than would be obtained if the energy liberated in the cell were all transformed into heat. This clearly indicates that some of the energy set free by oxidation is used for the repair of cell structure, chiefly perhaps, to further the endothermic reactions of synthesis. But the lowest caloric quotients are obtained with egg cells which are undergoing rapid division. This shows that the mechanical processes of development require energy.

The change in structure, that occurs after fertilization of the egg, has been shown by Warburg and others to increase oxidative metabolism. In some cases, it is more than doubled within a short period after fertilization. Even when development is started by artificial parthenogenesis so that no enzymes or other catalysts are introduced into the cells, oxidation speeds up as the structural changes occur.

These facts raise the question of what structures in the cell influence oxidation. For one thing, the cell membrane has been shown to be important. At the time of egg fertilization its membrane undergoes visible structural changes. The membrane becomes more distinct and becomes separated from the main mass of cytoplasm by a layer of transparent liquid. Further evidence is found in the fact that addition of alkali to the sea water surrounding such eggs causes a marked hastening of oxidative metabolism in spite of the fact that the alkali does not penetrate the egg. This experiment indicates that a change in hydrogen ion concentration can alter oxidation in the cell by an affect upon the cell membrane. It is also possible that the condition of colloidal particles, their state of subdivision, of hydration, etc., may be of importance. Membranes within the cell, dividing it into reaction chambers have been suggested as of significance in the regulation of cell oxidations.

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CHAPTER XXII

EXCRETION

In unicellular organisms, excretion occurs directly; that is, waste substances are put out of the cell through its limiting membrane into the surrounding medium. To some extent, excretion occurs in a more indirect way, namely, by the secretion of wastes into a vacuole which is later extruded from the cell.

In higher plants, excretion occurs chiefly through the leaves. Oxygen, when produced in excess during photosynthesis, becomes a waste product. Carbon dioxide, when produced in excess, is also a waste product. Both of these gases are excreted through the leaves. It is supposed that solid waste products of the plant are excreted directly through its roots into the soil water. Creatinine has been claimed to be a plant waste product which accumulates in the soil. In the main, however, plants are so predominatingly synthetic that solid wastes are not characteristic of the end results of their vital activities. The shedding of matured leaves and other parts of plants and the similar processes, by which plant structures are returned to the soil, permit bacterial decomposition largely to dispose of the solid wastes of plants.

In animals, excretion occurs through numerous different channels.

Paths of Excretion in Animals.—The following structures are concerned in the excretion of wastes from animal bodies: (1) The lungs and gills are the paths for excretion of gaseous wastes. The activities of these structures were described in a previous chapter. (2) The kidneys or some homologous nephritic organ, by secretion of urine, provide one of the chief paths for excretion. In all the higher animals, the urine, whether liquid, as in mammals, amphibians and fishes, or semi-solid as in birds and reptiles, carries nearly all of the solid wastes. The liquid urines are also the chief vehicle for the excretion of water. (3) The skin is an excretory organ. It is the path for excretion of some gaseous waste, in a few animals such as the frog. The sweat glands of the skin are excretory organs in some of the higher animals although their chief usefulness is found in heat regulation rather than in excretion. They certainly do not rank with the kidneys in importance as paths of excretion. (4) The liver, as already explained, effects some excretion, chiefly of cholesterol and bile pigments. The gall bladder also excretes cholesterol to a limited extent. (5) The intestinal epithelium excretes certain inorganic constituents which, if foreign to the body or present in excess of its needs,

may be regarded as waste substances. Rubidium, as representative of a foreign inorganic constituent, and calcium and magnesium, as representative of normal ones, are known to be excreted in part through the walls of the intestine. These structures can function so as to excrete water as in catharsis, induced by saline purgatives. All the digestive secretions convey some material into the contents of the alimentary canal and thus give opportunity for excretion of some of their constituents with the feces. The latter are composed mostly of material which is not truly excretory but consists of undigested food residues and bacteria that have grown in the intestine.

(6) Other secretions, in addition to those just mentioned, can also serve, in a minor way, as vehicles of excretion. The salivary, mammary and tear glands normally excrete traces of wastes and may excrete significant amounts of foreign substances (p. 463).

The kidney, as the chief organ for the excretion of solid wastes, requires especial discussion.

The Structure of the Kidney.

—The typical vertebrate kidney shows certain main divisions when seen in longitudinal section. (Fig. 165.) The outer portion of the kidney, that is, the part around its greater curvature, is called the cortex. The main mass of the kidney, enclosed by the cortex, is called the medulla. The medulla shows marked prominences, the pyramids, which contain the collecting and discharging tubules, and which project into the cavity which occurs in the inner curvature of the kidney. This portion is called the pelvis and is the region where the renal artery and vein connect with the kidney. The cavity in the pelvis receives urine as delivered by the tubules and connects with the ureter which conveys urine from the kidney to the bladder. The cortex is shown, by histological examination, to contain a very large number of nearly spherical structures known as the Malpighian bodies. These are enclosed by double walled capsules. Within the inner wall, (Fig. 166) is a tangled network of blood capillaries fed by a renal arteriole and emptying into a venule. This mass of capillaries is called a glomerulus. The membrane

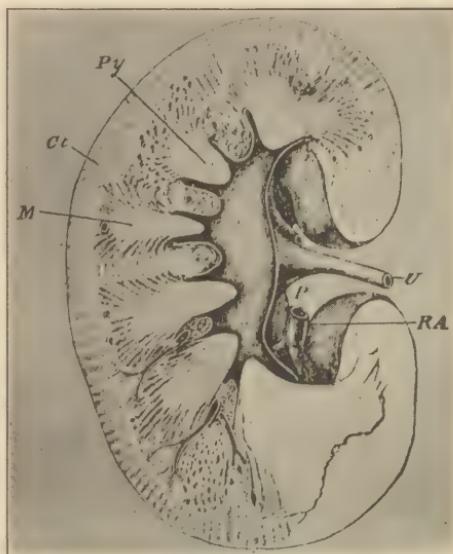


FIG. 165.—Longitudinal section of kidney.
Ct, cortex. M, medulla. Py, pyramids. P, pelvis. U, ureter. RA, renal artery. (From Huxley, "Lessons in Physiology," copyrighted, The Macmillan Co. Reprinted by permission).

which surrounds it is called Bowman's capsule. It forms the inner wall of the Malpighian body. This capsule is in close contact with the capillaries of the glomerulus and even dips into the irregular spaces between the capillaries. It is composed of flat structures similar to endothelial cells and is very thin. Thus, there are only two very thin membranes, that of the capillary wall and that of Bowman's capsule, between the blood in the glomerulus and the space of the Malpighian body. As this latter space forms the beginning of the kidney tubules which deliver urine into the pelvis of the kidney, the thinness of these membranes takes on special significance as indicating the possibility of filtration from the blood through the membranes. Bowman's capsule is thinner than are structures composed of typical cells. It

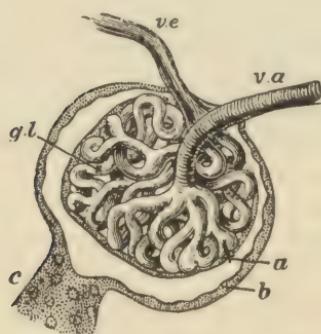


FIG. 166.—Glomerulus. *a*, capillaries. *b*, capsule. *c*, beginning of tubule. *gl*, glomerulus, consisting of a group of twisted capillaries and their investing membrane. *va*, arteriole. *ve*, venule. (From Huxley, "Lessons in Physiology," copyrighted, The Macmillan Co. Reprinted by permission).

appears, indeed, to form a syncytium because no definite cell boundaries can be recognized in it. This simplification of its structure is, perhaps, a further indication that it operates as a passive filtering membrane rather than as an active secreting structure. The urinary tubules which lead away from the capsules pursue a complex course partly in the cortex and partly in the medulla of the kidney. (Fig. 167.) A part of the tubule constitutes what is called the *loop of Henle*. This portion has thin walls. The differences in structure in the

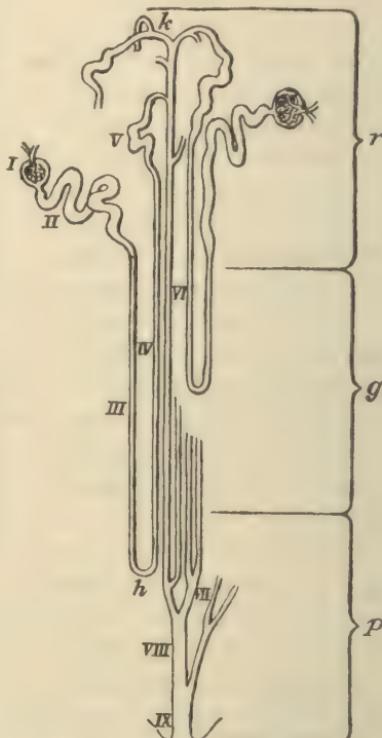


FIG. 167.—Diagram of kidney tubules. *r*, portion contained in cortex. *g*, in medulla. *p*, in pyramid. *I*, glomerulus. *II* to *IX*, various portions of the tubule. *h*, loop of Henle. (From Huxley, "Lessons in Physiology," copyrighted, The Macmillan Co. Reprinted by permission).

different parts of the kidney tubules have not been correlated with any known differences in function. The tubules are surrounded by net-works of blood capillaries which furnish ample opportunity for exchanges between the blood and the cells of the tubules. Beyond the loop of Henle further convolutions of the tubule lead into the collecting tubules which deliver the urine through larger tubules, of which they are branches, into the pelvis.

The Significance of the Kidney Secretion.—The uses which the kidney serves include: (1) The removal of non-volatile wastes; (2) the regulation of the hydrogen ion concentration. In this matter the respiration is also a significant factor, although without the regulative action of the kidneys, constancy of the C_H of the blood could not be maintained because of failure to eliminate any excess of non-volatile acids or bases. (3) The kidney removes the excess of certain nutrients, such as sugar and amino-acids, when their concentration in the blood is above normal. (4) The kidney also removes foreign substances such as iodides and other inorganic substances foreign to the blood. Similarly, organic foreign substances, such as vegetable and other pigments, drugs and the like, if not oxidized in the body, are commonly excreted in the urine. (5) The kidney regulates the osmotic pressure of the blood. This result is attained chiefly by regulation of the excretion of water and inorganic salts. In lower animals osmotic pressure is not regulated, but merely follows that of the surrounding medium.

Theories of Kidney Secretion.—Several theories have been proposed in explanation of the nature of the kidney secretion process.

1. A theory of physical filtration and diffusion was proposed by Ludwig. He suggested that filtration of the non-colloidal constituents of the blood occurred through the thin membranes of the glomerulus, thus admitting a dilute filtrate into the tubules. In the latter, water was absorbed according to his theory, so as to account for the concentration of urine. Urine is known to contain sodium chloride, urea, and several other substances in greater concentration than they show in the blood. Urea, for example, seldom if ever occurs in concentration above 0.05 per cent in normal blood but often constitutes as much as 2 per cent or more of the urine. Thus filtration in the kidney must be followed by concentration of the filtrate. Ludwig noted that there must be some exceptions to this theory in order to account for all the facts relating to the comparative composition of blood and urine.

2. A secretion theory was formulated by Heidenhain upon ideas earlier proposed by Bowman. According to this theory, water and salts were secreted by the glomerulus and the organic constituents of urine were secreted by the cells composing the walls of the tubules.

3. Cushny has proposed a theory which is essentially that of Ludwig but modified in certain particulars. According to this theory, all the

non-colloidal constituents of the blood plasma, together with a part of its water, filter through the glomerulus. As this solution proceeds through the tubules, some of the water, salts, glucose, amino-acids and other constituents, normally required for body uses, are reabsorbed through the walls of the tubules and thus restored to the blood. According to the theory, the absorbed material is comparable to a Ringer-Locke solution plus amino-acids and possibly some other constituents. Added to the colloidal constituents, this reabsorbed material restores the blood which leaves the kidney to its normal regulated composition. The reabsorption process requires the expenditure of energy in the kidney. Excretory products, such as urea, uric acid, etc., are not reabsorbed. According to this theory, the substances that are reabsorbed are never taken up completely. This explains why a trace of something which appears to be glucose is always present in normal urine. It also explains why some of the sodium chloride always appears in the urine even during extreme salt deprivation. Such useful substances are restored to the blood almost completely unless present in concentration above a certain limit. This limit is called the kidney threshold. It has a specific value, presumably, for each individual kind of nutrient or other useful blood constituent. For example, glucose is known to be excreted by the human kidney in significant amounts if present in the blood in concentration above 0.16 per cent. The excretion of glucose may occur at lower blood concentrations, owing to what is called a lowering of the kidney threshold for glucose. This occurs under some unexplained conditions and can be produced at will by administration of the drug *phloridzin*, a glucoside from the root bark of apple and other fruit trees. For most substances, however, the kidney threshold is constant and tends to maintain the concentration of each substance at the normal level in the blood. The resulting blood regulation is due, according to Cushny's theory, to the selective absorbing power of the kidney tubules.

4. Theories, that are modifications of these three have been proposed. As will be explained later, Cushny's idea of absorption without secretion in the tubules does not satisfactorily explain all the facts of experimental observation. These show that secretion plays some part in kidney activity. This is especially true of the tubules. Physiologists, who hold to the Ludwig theory of filtration in the glomerulus do not all accept Cushny's modification of this theory, but advance a still more complex one, namely, that both secretion and selective absorption can occur in the tubules.

The Action of the Glomerulus.—The structure of the glomerulus suggests that simple filtration occurs through its membranes. What conditions would have to be fulfilled if filtration occurs? The fact that, normally, only the diffusible constituents of the blood and not the colloidal ones appear in urine, indicates that semipermeable membranes are concerned in urine production. Such membranes permit osmotic pressure to

act. This would modify any process of filtration that might occur in the glomerulus. The osmotic pressure of the colloids of the blood would act against filtration and tend to draw water through the capsule into the blood. But mechanical pressure, that is, the blood pressure, would tend to force filtration through the capsule from the blood to the tubules. If, then, blood pressure is higher than the osmotic pressure of the blood colloids, filtration could occur. Both of these forces can be measured. Starling showed that the osmotic pressure exerted by the colloids of mammalian blood is about 30 mm. Hg. Correspondingly, urine secretion stops when blood pressure falls to about 30 to 40 mm. Hg. This fact has been established by numerous investigators. Any circumstance which changes the difference between the blood pressure and the osmotic pressure of blood colloids would change the rate of urine production in case filtration is concerned in the process. Such changes can be produced by injection of isotonic salt solution, thus diminishing the osmotic pressure of colloids by dilution of the blood. Such injections result in an increased rate of urine production. But if the colloid, gum arabic, is added to the salt solution in amounts sufficient to give it the same osmotic pressure, due to colloids, as that prevailing in blood, injection of this solution produces little or no increase in urine secretion. These experiments lend support to the filtration theory. Further support is found in the well-established fact that any increase in blood pressure in the kidney, other things being equal, increases the rate of urine production. Inasmuch as this result can also be obtained by dilution of the blood colloids without an increase of blood pressure, the difference between these two forces appears to be the significant factor. Other evidence in favor of the filtration theory has been obtained from the study of urine secretion in the frog. In this animal, the kidney possesses a double blood supply. The renal artery feeds the cortex of the kidney while the renal portal vein supplies the medullary portion. This means that the glomeruli do not receive blood from the renal portal system but only from the renal artery. When this artery is tied, urine secretion stops; but when the renal portal vein is similarly obstructed, secretion continues.

There are certain objections to the filtration theory: (1) Increased blood pressure, produced in the kidney by tying the renal vein, does not cause an increase of urine production but rather a decrease. (2) After cutting off the blood supply to the kidney, the resulting stoppage of urine secretion persists for some time, an hour or more, after the blood supply is restored to the kidney by removing the ligature from the artery. Both of these results indicate that, when oxygen is withheld from the kidney, its secreting mechanism suffers a temporary injury. It is hard to see how this result could be obtained upon a mere mechanical filter. (3) An obstruction to the outflow of urine through the ureter should, according to the filtration theory, cause a decrease in urine production; but experi-

mentally, this is not found to be the case. A slight obstruction of the ureter may actually result in an increased rate of urine production even against the opposing pressure thus produced in the ureter.

These objections show that filtration alone could not account for urine production but none of them disprove the possibility that a filtrate from the glomerulus enters the tubules to be modified in them by processes that include both absorption and secretion. If such filtration occurs, the filtrate is composed of blood plasma constituents minus the colloids.

The Action of the Tubules.—Urine itself does not resemble a filtrate from the blood plasma. Urea and some other waste substances are much more concentrated in urine than in blood, while useful nutrients, such as amino-acids and glucose, are much less concentrated in urine or may be entirely absent. These substances are known to be present in diffusible form in the blood. These facts point to the selective absorbing power and probably to the secretory activity of the kidney tubules. It is impossible to say whether absorption alone, secretion alone or a combined action of both these processes occurs in the tubules. If the glomerular filtration theory is accepted, absorption in the tubules must also be conceded in order to account for the concentration of urea and NaCl in the urine and the nearly complete absence of glucose and amino-acids. But much evidence is available to show that secretion occurs in the tubules. Some of this evidence follows: (1) Several investigators, especially Bainbridge and his co-workers, have shown that when the renal artery of the frog is ligated, injection of urea causes secretion of urine. Under these conditions, as explained above, only the medullary portions of the kidney, including the tubules, receive a blood supply. Thus, these experiments show that the tubules can secrete urea and water. (2) When the ureters of birds are ligated, uric acid is found in the kidney tubules but never in the glomerular capsules. Microchemical evidence also indicates that secretion of uric acid occurs through the walls of the tubules. (3) Indigo carmin, injected into the circulation, is excreted by the kidney. Heidenhain has shown that when urine secretion is slow, enough of this dye accumulates in the kidney to permit its detection after the kidney has been irrigated with alcohol in order to fix the dye in the tissue cells. He found the indigo carmin in the tubule cells but never in the glomeruli. This indicates that a foreign substance can be excreted through the tubules without passing through the Bowman capsules. (4) By the use of indicators, such as acid fuchsin and neutral red, which can penetrate living cells, the medullary portion of the kidney is found to show an acid reaction while in the case of the frog, at least, the glomeruli are not acid. This suggests that the excretion of potentially acid substances is a result of secretion on the part of the tubular cells. (5) Numerous investigators have described microscopic appearances which indicate secretion in the tubular cells. Vesicles or secretion vacuoles are

pictured and their appearance indicates that they discharge from the cells into the lumen of the tubules.

On the whole, it seems impossible to deny that the tubular cells can actively secrete, however much their power to absorb water, salts and other useful substances from the tubules may be emphasized.

The Work Done by the Kidney.—Energy is expended during urine secretion. This is shown by the increase of oxidative metabolism during increased activity of the kidneys under certain conditions. The measurement of the increased metabolism can be made by analyses of samples of blood from the renal artery and vein in order to determine the O_2 consumption and CO_2 production in the kidney. If the rate of oxidation, thus measured, is compared with the amount and composition of the urine which is secreted during the same period of observation, results show that to produce a larger amount of urine of a given composition or to produce the same amount of urine which is more concentrated in certain constituents, requires increase of oxidative metabolism, that is, expenditure of more energy. In some cases of secretion, work is done in the formation of new compounds. In the case of the kidney, expenditure of energy for this purpose is a minor matter, in that synthesis of new compounds occurs to only a slight extent. Most of the work accomplished by the kidney is the concentration of urine. This amounts, practically, to the conservation of water and other useful constituents. Concentration of any solution requires heat, or its equivalent in some other form of energy, to separate the solvent from the solutes. To produce this effect in the kidney, the work might take the form of withdrawal of water and certain selected solutes from the kidney tubules or that of the secretion of water and certain wastes from the blood into the tubules. Either form of work would require energy. Studies of the work done by the kidney, therefore, fail to give a decisive answer to the long disputed question of the nature of the process of urine production. Certain of these studies do furnish some aid in answering the question. For example, the work of Barcroft, Cushny and others has shown that injection of NaCl solution or Ringer solution does not increase the O_2 consumption of the kidney although it causes a very great increase in the volume of urine produced. This proves that the kidney can excrete water and physiological salts without expenditure of energy. In contrast to these results, is the effect of Na_2SO_4 . If this is injected into the blood, it also causes a marked increase in the rate of urine secretion, but this increase is accompanied by a corresponding rise in the O_2 consumption of the kidney. A satisfactory interpretation of these results is difficult because they might be explained either as an indication of filtration through the glomerulus followed by concentration in the tubule or as an indication of the secretion of Na_2SO_4 in both the glomerulus and the tubule. Further similar work has given more weight to the filtration

theory. It has been shown that when the kidney is poisoned with mercuric chloride, its consumption of O_2 is very much decreased. But under these conditions, NaCl can still cause an increased urine production although Na_2SO_4 cannot. This clearly points to the idea that water and NaCl, at least, can be excreted by the mere mechanical process of filtration without any expenditure of the form of energy that is required for secretion. The force needed for filtration is blood pressure and this is furnished by the heart beat and not by kidney metabolism.

Diuresis.—Any marked increase in the rate of urine secretion is called diuresis. Substances which produce this effect are called diuretics. Urea has marked diuretic action. The rate of its excretion is directly proportional to its concentration in the blood, over a wide range of concentrations such as have been obtained by administration of excessive amounts of pure urea. Excretion of water tends to run parallel with that of urea so that, if sufficient quantities of water are available to the organism, the volume of urine produced tends to be proportional to urea excretion. Inasmuch as urea is the chief end product of protein catabolism in mammals, a mild diuresis accompanies a high protein diet. Glucose also causes diuresis when present in the blood beyond the physiological limits. This effect is seen in the enormous volumes of urine, sometimes as much as 5 liters or more per day, excreted by diabetic patients. The diuretic effect of salts, such as NaCl and Na_2SO_4 was discussed above. Many other salts have a marked diuretic action. The acetates of sodium and potassium are especially effective and are included among the salts which are used for the production of diuresis in medical practice. Several drugs are known to have a marked diuretic action. Caffeine is one of the most notable.

The immediate cause of diuresis appears, in some cases, to be an increased water content of the blood. At any rate, substances, which by osmotic pressure would tend to draw water from the tissues into the blood stream have marked diuretic action. This is the case with inorganic salts, urea and glucose when they are present in the circulation in unusually high concentration. In other cases, diuretics may act more indirectly by causing an increased blood flow through the kidney. This is brought about in the main by the action of the vasoconstrictor nerves. Vasoconstrictor effects are able to alter kidney secretion by changing the relative dilatation or constriction of the renal arteries and arterioles or by changing the general level of blood pressure in the systemic circulation. In these ways, the nervous system exerts effects upon the kidney and may even cause a nervous diuresis under emotional strains or a marked decrease in urine secretion. Direct nervous control of the kidney cells has never been demonstrated.

The Volumes of Urine Produced.—In aquatic animals, which have no need to conserve water, the volume of urine produced is not apparently

under strict regulation. In the frog, for example, large volumes of very dilute urine are commonly produced. In land forms, however, the excretion of water must be strictly regulated in accordance with the water intake. The intake of water includes the moisture content of solid foods and the water produced by oxidative metabolism. Under most normal circumstances, the volume of the urine is determined by the amount of water ingested and produced. This fact is not always apparent as certain factors interfere with this correlation. One of them is concentration of the blood by the passage of water into the tissues. Another is a decrease of blood flow through the kidneys. A third is the secretion of sweat. In short, any condition which cuts down the amount of water available for excretion decreases the volume of urine produced. Moreover, abnormal circumstances, such as diabetes, cause increased urine secretion as an accompaniment of sugar excretion. Also, diabetes insipidus causes the production of large volumes of urine without any tendency to excrete sugar. Volumes of urine as high as 15 to 25 liters per day are observed, and one case has been reported as producing 43 liters in one day, or nearly 1800 cc. per hour. In diabetes insipidus, the kidney merely fails to hold back water as it normally does. Any tendency of the blood to become concentrated, whether due to sweating, to excess of water excretion into the urine or to water deprivation is accompanied by thirst which tends to insure a degree of water ingestion proportional to its excretion.

The semi-solid urines of birds and reptiles have this consistency as the result of water absorption which occurs largely in the cloaca. Their urines are probably always liquid as they leave the kidney.

The General Characteristics of Urine.—The urine of mammals varies greatly in its proportions of water and solids. In man, for example, solids of the urine may vary from about 0.7 per cent to about 11 per cent even under strictly physiological conditions. The specific gravity varies accordingly. Figures as low as 1.003 and as high as 1.040 are not infrequently observed, although the majority of human urines have a specific gravity between 1.018 and 1.024. The variability in the relative water and solid content is the result of the regulated action of the kidney. Any increase in water ingestion causes a corresponding increase in urine dilution, while profuse sweating during water deprivation causes the secretion of a urine of comparatively low water content and high specific gravity. The high sugar content of some diabetic urines gives them a high specific gravity, sometimes as high as 1.055.

The reaction of urine is also subject to great variations, determined by the relative ingestion of potentially acid or basic substances. Herbivores usually excrete an alkaline urine of pH slightly above 7. This is due to the basic salts of vegetable foods. Carnivores excrete an acid urine (pH about 5). This is due chiefly to the phosphates and sulphates

derived from the oxidation of protein foods. Human urine is commonly on the acid side of neutral. The pH is usually between 4.8 and 7, with an average of about 6. The figures are higher for vegetarians who may even secrete an alkaline urine. The figures just given apply to mixed samples of 24-hour urines. During limited periods the urine may show marked variations from the average for the day. After a full meal, human urine generally shows what is called the alkaline tide, that is, the urine is alkaline during the two or three hours after eating. This is attributed to the withdrawal from the blood of the material required for the secretion of the HCl of the gastric juice. This production of an alkaline urine prevents any disturbance of the acid-base balance of the blood which would otherwise occur during the secretion of gastric juice. Another cause of alkalinity in urine is bladder infection. Many bacteria are able to ferment urea with the production of ammonia. This is sufficient to render the urine alkaline when such bacteria are present in the bladder. The same fermentation process occurs in urine after it has been voided so that it grows alkaline if kept without an antiseptic.

The relative transparency, which ordinarily prevails in the urine of most mammals, may be replaced by a marked turbidity or by the formation of distinct sediments. In human urine, this is normally due either to phosphates, which separate out as calcium and magnesium phosphates in an alkaline urine, or to ureates, which tend to separate as ammonium ureate when uric acid is abundant in the urine or as acid ureates when the urine is distinctly acid. Other sediments may occur pathologically.

The osmotic pressure of urine varies as widely as does its relative content of solids. This is chiefly the result of the variation in the proportions of salts and water excreted. By the cryoscopic method, Δ is found to vary in human urines from 0.3 (freezing point $-0.3^{\circ}\text{C}.$) in very dilute urines to 3 (freezing point $-3^{\circ}\text{C}.$) in concentrated urines. The majority of results on normal, human urines are between 0.51 and 0.62. The variability of the osmotic pressure of the urine shows the effectiveness of the kidneys for the maintenance of the regulated osmotic pressure of the blood.

The Composition of Urine.—Urine constituents may be classed as the nitrogen-containing wastes, the nitrogen-free organic compounds and the inorganic salts. The following table gives a general idea of the relative abundance of the chief constituents of normal, human urine. The table includes only those substances which are readily determined quantitatively. The values given are based upon a limited number of analyses and do not show the average composition of human urine although they are fairly representative results.

It should be understood that all these quantities vary widely with different individuals and with the same individual under different diets and conditions of normal living. They vary still more widely under

abnormal and pathological conditions. The constituents shown in the table would account for about 99 per cent of most normal human urines.

REPRESENTATIVE COMPOSITION OF NORMAL HUMAN URINE

Volume for 24 hours.....	1250 cc.
Specific gravity.....	1.019

	Weight, grams	Approximate per cent
Water.....	1212.0	95.1
Solids.....	61.7	4.9
Nitrogen-containing constituents:		
Urea.....	28.5	2.28
Creatinine.....	1.7	0.13
Ammonia, computed as NH ₃	0.7	0.05
Uric acid.....	0.65	0.05
Hippuric acid.....	0.6	0.04
Indican, indoxyl potassium sulphate.....	0.01	0.0008
Allantoine (not always present).....	0.005	0.0004
Creatine (not usually present in urine of healthy adults but may occur in traces)		
Nitrogen-free organic constituents:		
Glucose or similar carbohydrate.....	0.7	0.05
Aromatic oxyacids.....	0.05	0.0004
Oxalates, as oxalic acid.....	0.015	0.001
Acetone + aceto-acetic acid.....	0.01	0.0008
Inorganic constituents:		
Chlorides, as NaCl.....	11.0	0.90
Phosphates, as P ₂ O ₅	2.2	0.18
Sulphates, as SO ₃	1.7	0.14
Potassium.....	1.6	0.12
Calcium.....	0.2	0.01
Magnesium.....	0.2	0.01
Iron.....	0.005	0.0004

Other substances are found in traces in human urine. Among these are the purine bases, guanine, adenine, xanthine and hypoxanthine, which are closely related, chemically and physiologically, to uric acid. There are also other purine bases, the methylated purines, such as epiguanine, paraxanthine, heteroxanthine and 1-methylxanthine, which are derived from methylated purines of certain vegetable substances. Amino-acids are detected in minute traces in normal human urine. Proteins also occur in amounts too small to be detected by the ordinary protein tests. The proteins probably are not added to normal urine by secretion in the kidney but are derived from the cells which slough off from the linings of the urinary passages into the urine. Several sulphur

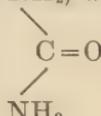
compounds, in addition to indican and inorganic sulphates, are found in traces in urine. These include chondroitin sulphuric acid, thiocyanates, taurine derivatives and ethereal sulphates (p. 493) similar to indican. Urine always contains substances of a carbohydrate character, such as glycuronates (p. 607), in addition to glucose. Pigments, such as urochrome and urobilin, give urine its yellow or brownish color.

Under pathological conditions, many other substances may be found in urine. Proteins of the blood (serum albumin, serum globulin, oxyhemoglobin) may occur in urine. Serum albumin is the one most commonly detected as a diagnostic sign of certain renal disorders and of some other diseases. Its presence in the urine is spoken of as **albuminuria**. Other proteins, such as nucleoprotein, proteoses and peptones, occur less frequently in urine. Bile constituents, the pigments and the bile salts, occur in the urine as the result of any obstruction to the outflow of bile from the liver into the intestine. Carbohydrates, such as pentoses, lactose, galactose and fructose, appear in the urine under any conditions which cause the accumulation of one or more of these substances in the blood. Lactose appears as the result of failure to remove milk from the lactating mammary gland. During defective carbohydrate metabolism, glucose, acetone, aceto-acetic acid and β -hydroxybutyric acid are found in significant amounts. All of these except β -hydroxybutyric acid commonly occur in faint traces in normal urine. Pus and material cast off from the tubules of the kidney, the so-called "kidney casts," form a sediment in the urine during some infections that involve the kidney. Foreign substances, such as drugs, abnormal pigments, arsenic, lead and mercury, are excreted in the urine. The qualitative and quantitative analysis of urine is obviously of great value in the diagnosis of many disturbances in human metabolism and in the study of normal metabolism in all animals. The work of Folin and that of S. R. Benedict has developed the modern effective methods of urine analysis. Their researches, together with the work of others who have largely used their methods, have thrown much light upon the problems of the metabolic significance of the composition of urine in health and disease.

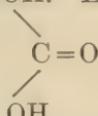
All the nitrogenous substances representing the end products of protein metabolism are excreted in urine. In mammals, amphibians and fishes, urea is by far the most abundant of these end products. In birds and reptiles, uric acid is the predominating one. The end products of protein metabolism in invertebrates have not been satisfactorily studied. Among the vertebrates, several constituents, other than urea and uric acid, vary as to their abundance in the urine of different species. Hippuric acid is one of the most abundant constituents of the urine of horses and cattle, though sometimes present in the merest traces in human urine and may even be entirely lacking from the urine of carnivores. Allantoin is comparatively abundant and uric acid very scanty in the urine

of all mammals except man and the anthropoid apes. Creatin, though often absent from human urine, is always found in the urine of children and in that of starving persons, diabetics and those suffering from certain other derangements of metabolism. These differences between species and individuals are due in part to differences in diet and in part to variations in the nature of metabolic processes. The significance of the different constituents is discussed below in connection with their chemistry.

Urea.—Urea has the formula NH_2 , which shows it to be the diamide

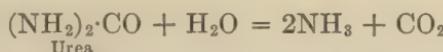


of carbonic acid, OH . Except during certain pathological conditions,



urea contains 60 to 90 per cent of all the nitrogen excreted in human urine. During very high protein feeding, urea may contain as much as 95 per cent of the total nitrogen of the urine. But when ammonia is very abundant in the urine, urea may constitute a correspondingly low proportion. The proportions of urea and ammonia were studied by Folin.

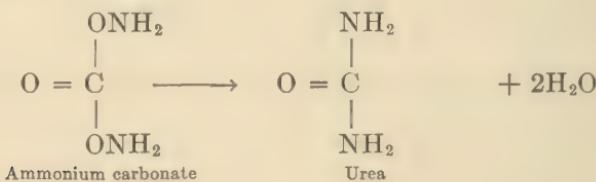
Urea crystallizes in long, colorless, rhombic prisms which are very soluble in water and in alcohol but are insoluble in ether or in chloroform. Urea can be crystallized out of an alcoholic extract of highly concentrated urine. When dry urea crystals are heated, they melt and decompose with the liberation of ammonia. The mass, which is left after such heating, contains biuret, $\text{H}_2\text{N}\cdot\text{CO}\cdot\text{NH}\cdot\text{CO}\cdot\text{HN}_2$, which is produced by the union of two urea molecules with the elimination of one molecule of ammonia. Biuret is detected by the violet color, which it gives in alkaline solutions containing copper. This is similar to the color reaction, called the biuret test, which is frequently employed for proteins. Urea reacts with hypobromites, such as NaOBr , to form free nitrogen and carbon dioxide. Measurements of the nitrogen given off during this reaction afford an approximate method for the quick estimation of urea in urine. But more accurate methods have been based upon the cleavage of urea to form ammonia. This cleavage is obtained in several ways. The most satisfactory methods employ an enzyme, **urease**, which has been found to occur in the soy bean and which is specific in its catalytic effect upon the reaction:



Measurements of the resulting ammonia permit the calculation of urea in

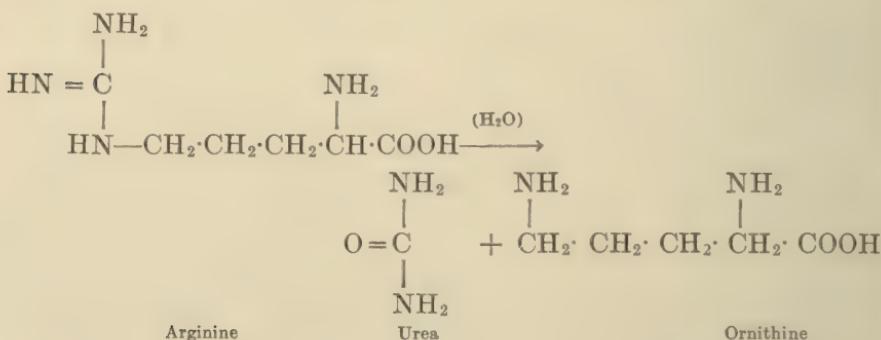
urine, provided the ammonia content of the same specimen is determined for the necessary correction.

The origin of urea in the body is the catabolism of amino-acids. Several of these, when added to blood perfused through the liver, have been shown specifically to give rise to urea. Indirect evidence indicates that all the organs of the body can effect this change. Presumably, the amino-acids are first deaminated, that is, deprived of their amino groups which give rise to ammonia. The latter is synthesized with carbonic acid to form urea. Probably, ammonium carbonate is first formed and then converted to urea.



Ammonium carbonate, when perfused through the liver, is readily converted into urea. As the major part of the amino-acids metabolized in the body undergo deamination, the resulting ammonia causes urea to become the chief end product of protein metabolism in many animals. In birds and reptiles, the urea which is produced, appears to be synthesized with other compounds (chiefly lactic acid) to form uric acid.

A small part of the urea produced in the body arises in a more direct way. The amino-acid, arginine, yields urea under the influence of the enzyme called **arginase**. It has been found in liver and in intestinal walls but appears to be lacking in muscle. This enzyme splits arginine so as to liberate urea and ornithine.

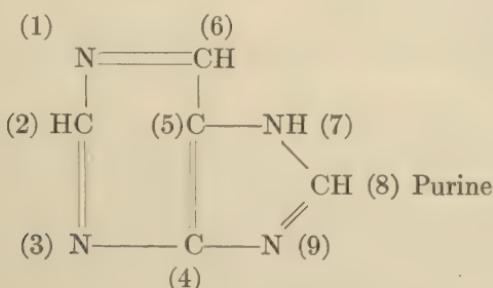


Ammonia.—Ammonia occurs in urine in the form of various salts, such as chlorides and ureates. In order to measure the amount of ammonia, it is liberated by an alkali, driven off from the urine by a current of air and collected by causing the air to pass through a measured

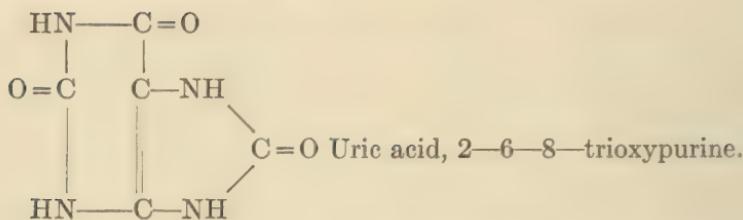
quantity of standard acid. Sodium carbonate is employed to liberate the ammonia because this alkali is not sufficiently strong to cause the conversion of urea into ammonia. The ammonia, collected in standard acid, can be measured by titration of the excess of acid in order to find how much of the acid was neutralized by the ammonia evolved from the urine. Ammonia may also be determined by causing it to develop a color reaction with Folin's modification of Nessler's reagent which is an alkaline solution of mercuric iodide. Ammonia develops a yellow color with this reagent. The intensity of this color, measured in a colorimeter, by comparison with a color similarly developed in a standard solution of an ammonium salt, permits computation of the ammonia driven off from the urine.

The origin of ammonia in the body is, primarily, the deamination of amino-acids. In so far as the ammonia thus liberated combines with anions other than $-CO_3$, it tends to be excreted in the urine without being converted into urea. This means that ammonia is one of the safeguards of the body against acidosis. The amount of HCl secreted into the gastric juice appears to influence the amount of ammonia in the urine. Presumably HCl of the gastric juice is neutralized, in part at least, by ammonia. During derangements of metabolism that cause defective oxidation and consequent acidosis, ammonia in the urine is greatly increased. This also is the result of the use of ammonia by the body to neutralize non-volatile acids. In this case, these acids are produced by defective oxidation.

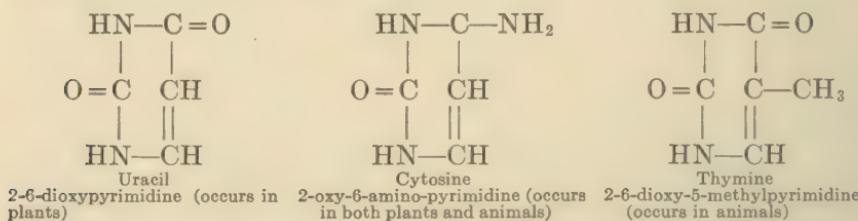
Uric Acid, the Purine Bases and Allantoin.—Uric acid is one of the purine compounds. These substances have been systematically classified through the work of Emil Fischer. He has shown how they are all related to one another in that they are derivatives of purine. Purine itself apparently does not occur in nature but has been synthesized by Fischer. It has the following formula:



The numbers in parenthesis are useful for reference in description of purine derivatives. Thus, uric acid is described as 2-6-8-trioxypurine. Its formula is:



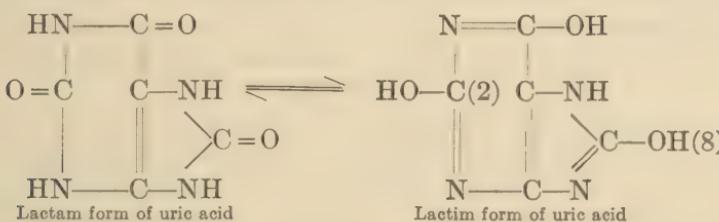
This formula shows that uric acid contains two urea residues joined to a chain of three carbon atoms. The formula also shows that the uric acid molecule contains the pyrimidine grouping, as is seen by comparison with the formulas of the three pyrimidine bases that occur in nature namely, *uracil*, *cytosine* and *thymine*:



No evidence has been obtained to show that pyrimidines ever contribute to the production of uric acid; but purines and pyrimidines probably have a similar origin in plants and animals. At any rate, all living things are able to synthesize both purines and pyrimidines from foods that apparently contain no substances related to either of these groups of compounds. All growing things produce nucleoprotein which contains purines and pyrimidines, yet the simple foods of plants and the milk or egg substance, which nourishes very young animals, are devoid of any significant amount of either purines or pyrimidines. These facts lend significance to the presence of the urea groups and the chains of three carbon atoms in purine and pyrimidine substances, because the urea group can be derived from protein decomposition and the chain of three carbon atoms arises in many ways during metabolism, as explained in the preceding chapter.

The Properties of Uric Acid.—The name uric acid is somewhat misleading because this substance does not have typically acid properties. It possesses no carboxyl ($-\text{COOH}$) group and does not dissociate hydrogen ions to any significant degree in water. Indeed, uric acid, unlike most organic compounds that are called acids, is almost completely insoluble in water. Its name was chosen before its structure was understood and refers to the tendency of uric acid to form "salts" with bases. The resulting ureates are many of them soluble in water. This is especially true of the lithium, sodium and potassium compounds. They occur in two series, the mono-basic or acid salts and the di-basic or neutral salts.

The latter are much more soluble than are the acid series. Ammonium ureate and the ureates of calcium, magnesium and of all the heavy metals are comparatively insoluble. The ammonium, calcium and the acid sodium ureates often separate out of urine as amorphous sediments or as crystals which may be identified by their characteristic form. The explanation of this salt formation in spite of the absence of carboxyl groups, is given by the theory that uric acid undergoes tautomeric rearrangement from its so called lactam form to the lactim one.



This reversible reaction is apparently influenced by the C_H . of the solution. In the lactim form, the H of the —OH groups in the (2) and (8) positions are replaceable by basic groups or by metals. The H of the third —OH group, the one in the (6) position does not appear to be replaceable.

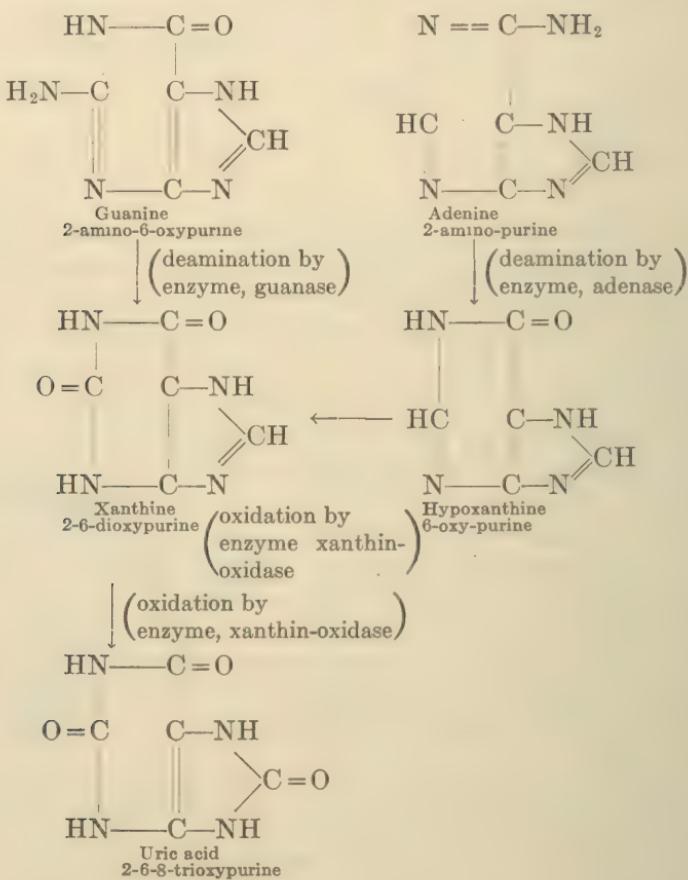
The identification of uric acid is accomplished most easily by acidification of its solutions or of urine. It then crystallizes out in characteristic forms. The ones obtained from urine are colored red by urine pigment and show some modification of the so-called whetstone form. (Fig. 168.) Uric acid shows several color reactions. One of them is the murexide test, a purple color with ammonia after the uric acid has been evaporated with nitric acid. Another color reaction is given with phosphotungstic salts in alkaline solution. This yields a proportional to the amount of uric acid deep blue color of an intensity acid present. Because of this proportionality, the reaction has been used as the basis for all of the newer methods for quantitative determination of uric acid in urine, blood and tissues. The uric acid is first separated as one of its insoluble salts. It is then dissolved in alkaline solution, is treated with the phosphotungstic reagent and sodium carbonate and is then compared, colorimetrically, with a standard



FIG. 168.—Various forms of uric acid crystals obtained from urine.

solution of uric acid in which the blue color has been developed by a similar treatment.

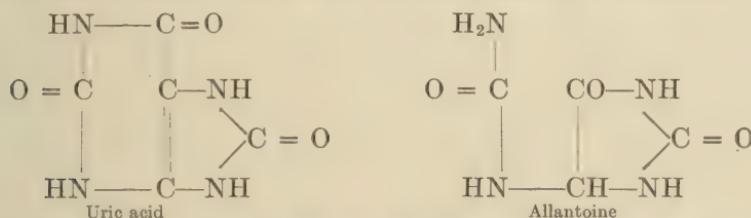
The Purine Bases and Their Relation to Uric Acid.—In addition to uric acid, the purine derivatives include the purine bases. The ones which are chemically and physiologically in close relation to uric acid are guanine, adenine, hypoxanthine and xanthine. Their structural formulas show their relationship to uric acid and to each other. The arrows, shown in the following scheme, serve to indicate how the animal organism can transform these substances by metabolic processes which are indicated in the scheme.



The origin of the purine bases is primarily the catabolism of nucleoproteins or, more strictly speaking, of nucleic acid. Nucleic acids are hydrolyzed by certain enzymes to yield guanine and adenine among their hydrolytic products. By deamination and oxidation as represented in the above scheme, uric acid is produced. A similar result is also obtained in the body by a slightly different series of reactions. In these, guanine

and adenine are subjected to deamination before they are liberated from other parts of the nucleic acid complex. On this account, they appear as hypoxanthine and xanthine, especially in muscle tissue, when they are liberated by hydrolysis. They subsequently undergo the usual oxidation to uric acid. Animal metabolism tends to carry these various reactions far enough to produce uric acid from most of these purine bases that arise in the body. On this account, adenine, guanine, hypoxanthine and xanthine appear in mere traces in the urine. A disturbance in this metabolic process has been observed in pigs. It results in a failure to deaminate guanine so that this base accumulates in the tissues and gives rise to what is called guanine gout with crystals of guanine forming in the joints.

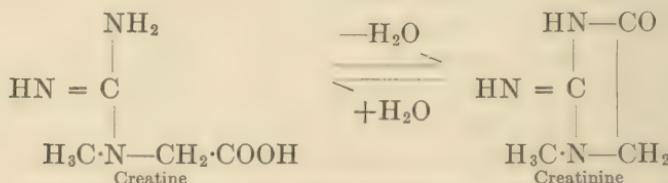
Allantoine and Its Relations to Uric Acid.—The action of uricase (p. 665) causes oxidative destruction of uric acid in all mammals except man and the anthropoid apes. With the exception of one breed of dogs (p. 591) all mammals, used for metabolism studies, are found to have only small quantities of uric acid in the urine. Allantoine is the chief product of purine metabolism in these animals. It is known to be formed by the action of uricase on uric acid and can be obtained *in vitro* when uric acid is mildly oxidized by potassium permanganate in alkaline solution. Certain other oxidizing reagents produce the same result. All these facts indicate that allantoine is derived in the body from oxidation of uric acid. The formula of allantoine shows its close relationship to uric acid.



The name allantoine was chosen because this substance was first discovered in the allantoic fluid. It is more abundant in bovine than in human allantoic fluid. The fact that the human body lacks the enzyme, uricase, and does not transform uric acid into allantoine, helps to explain the occasional accumulations of uric acid in human blood and tissues. When it crystallizes in the joints, it gives rise to gout and arthritis. It also crystallizes, at times, in the kidney, forming urinary calculi (kidney stones). The small amount of allantoine in human urine doubtless comes from meat of the diet.

The methylated purine bases, listed above as present in human urine, are derived chiefly from caffeine (1-3-7-trimethyl xanthine) of tea and coffee and from theobromine (3-7-dimethyl xanthine) of cocoa and chocolate. These bases are excreted after slight modification. Both of them may be excreted unchanged when consumed in large amounts.

Creatine and Creatinine.—Creatine is methyl-guanidine acetic acid and creatinine is its anhydride.



These formulas show the close chemical relations of these compounds to each other. Their behavior confirms this relationship. Creatine is converted quantitatively into creatinine by boiling in strongly acid solution. The reverse change occurs when creatinine is mildly heated in an alkaline solution. Under these conditions, the two are mutually interconvertible and the reaction between them comes to an equilibrium at which creatine is much more abundant than creatinine.

The occurrence of these two substances in urine is not the same; for while creatinine is present in considerable amounts in the urine of man and of all animals used for laboratory purposes, creatine is commonly absent from the urine of normal, adult animals and appears only during growth, starvation, defective carbohydrate metabolism, wasting diseases or during the use of a diet which contains much creatine (high meat diet). The amount of creatinine in the urine of a normal individual is surprisingly constant. Although it shows some fluctuations, these are within much narrower limits than are those of other urine constituents. The amount of creatinine excreted per day varies considerably in different individuals. For any one person, the so called creatinine coefficient may be computed. This quantity is obtained by dividing the total number of milligrams of creatinine in the day's urine by the body weight expressed in kilograms. For a given individual, the creatinine coefficient may remain practically constant over long periods and this in spite of great changes in the diet. The constant excretion of creatinine and the nearly constant lack of excretion of creatine is in marked contrast to the occurrence of these two substances in blood and tissues. Creatinine is found very sparingly and possibly not at all, in free form, in the blood and tissues; while creatine, in some tissues at least, appears to be comparatively abundant. There is some evidence to indicate that even creatine is not present in blood and tissues in a strictly "free" condition. It is found, for example, that creatine of the blood is contained normally in the corpuscles and does not occur in the plasma except during the unusual conditions which lead to the presence of creatine in the urine. Also the creatine of muscle is much more abundant than that of blood and yet muscles can take creatine from the blood when present there in very low concentration. This indicates that creatine is held in muscle cells in some

non-diffusible complex. It must be a very loose combination, however, for creatine is completely extracted from dead muscle substance by water.

The properties of creatine and creatinine include high solubility in water. Creatine, as prepared from muscles, crystallizes in truncated prisms. Creatinine, as prepared from the urine, separates with zinc chloride in characteristic rosette-shaped crystals composed of creatinine zinc chloride. One especially useful color reaction is given by creatinine. With picric acid in alkaline solution, it gives a reddish compound which has an intensity of color proportional to the amount of creatinine present. This reaction is therefore used as the basis of a quantitative method for the colorimetric determination of creatinine. Inasmuch as creatine can be converted into creatinine by heating in acid solution, creatine can also be determined by means of the same colorimetric method.

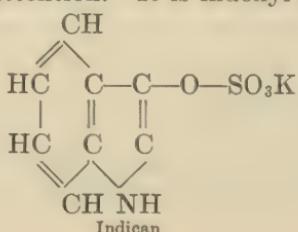
The origin of these substances in the animal body is still far from certain, although many facts have been established by the extended researches of numerous investigators of creatine and creatinine metabolism. The close chemical similarity of these two compounds suggests that they have the same origin. But this idea is not entirely substantiated by observations upon the effects of feeding these substances to man or to animals. Creatine, when fed in moderate amounts, largely disappears as though it were absorbed and retained by the tissues. A small proportion of it may appear in the urine. Administration of excessive quantities of creatine causes the excretion of a larger proportion of it than when smaller amounts are fed. This is as if the tissues showed a certain saturation limit beyond which they are unable to take up more creatine. In any case, creatine that is fed is excreted to only a slight extent as creatinine. On this point observers disagree, but in any case the conversion of food creatine to creatinine does not occur extensively in the body. This does not necessarily exclude the possibility that creatine, which has actually been incorporated in the cells, may be converted into creatinine by processes which are regulated so as to maintain creatinine production nearly uniform in rate, in spite of creatine feeding. The comparative abundance of creatine in certain tissues, especially in muscle, suggests that it is a forerunner of creatinine. Creatine constitutes 0.3 to 0.5 per cent of fresh muscle and is the most abundant nitrogenous compound of a non-protein character in muscle. On this account, numerous attempts have been made to connect creatinine excretion with muscular metabolism. Creatinine of the total day's urine is not increased by muscular work, though a temporary increase followed by a compensatory decrease has been observed. Moreover, activity does not increase the content of creatine in muscle, at least according to some observers. Nevertheless there appears to be some connection between creatinine production and muscle metabolism. This is shown by the fact that the creatinine coefficient is higher for persons with efficient,

trained muscles, especially if they show a high degree of muscle tonus than in the case of persons with poor muscular development. Creatinine, when fed or injected is excreted in the urine to the extent of about 80 per cent of that introduced. Some of it (about 20 per cent) disappears. It is not excreted as ammonia or urea. Apparently, it is retained in the tissues in some form, possibly as creatine. But, on the whole, proof is not adequate to show that creatinine is formed in the body from creatine nor has the occurrence of the reverse change from creatinine to creatine been satisfactorily established.

The significance of the appearance of creatine in the urine (creatinuria) during growth and during certain diseases and disturbances in metabolism requires further investigation; but one point, which helps to explain it, has been established. It has been shown that, during starvation, creatine excretion can be decreased or even stopped by the feeding of carbohydrate food. Moreover, when sugar utilization becomes possible in a diabetic because of recovery from the disease, creatine disappears from his urine. These observations indicate that the metabolism of creatine, a nitrogenous compound, is in some way connected with carbohydrate metabolism. This adds further emphasis to the generalization discussed in the preceding chapter, namely, that cells do not utilize their food substances by means of chemical reactions that are independent of one another.

The forerunners of both creatine and creatinine in the body are as yet unknown. Attempts have been made to connect the amino-acid, arginine, with the production of creatine or creatinine. This compound has been used in such experiments because it contains the guanidine group in its molecule. All such attempts have been without success. It seems probable that any arginine, which is not utilized in the body for the synthesis of proteins, is destroyed by the enzyme arginase with the production of urea (p. 638) rather than of a guanidine-containing substance. Several different syntheses of creatine and creatinine have been effected *in vitro* but none of them have been shown to occur in a corresponding way in the animal body. Whatever may be its origin the constant production of creatinine, irrespective of diet, indicates that it is the result of some metabolic process which goes on unfailingly as though it were an indispensable part of animal life.

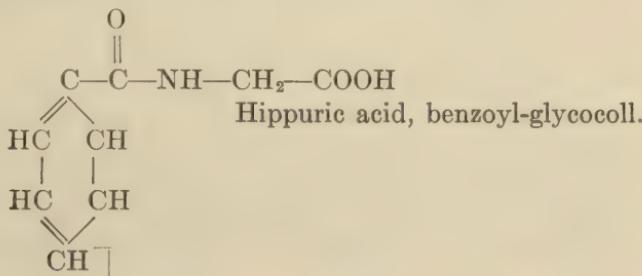
The Ethereal Sulphates.—The urine contains small quantities of organic sulphate compounds known as the ethereal sulphates. Of these, indican has received the most attention. It is indoxyl potassium sulphate.



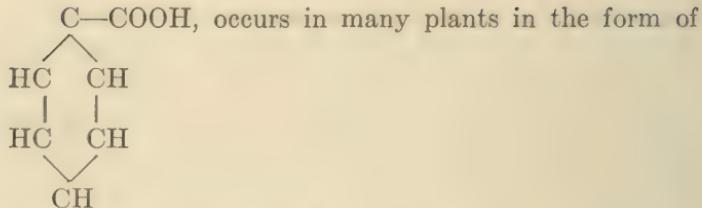
It contains the indole grouping which comes from the indole produced by intestinal putrefactive bacteria acting upon the amino-acid, tryptophane, of protein digestion products. To some extent, indole appears in the urine in combination with glycuronic acid as well as sulphuric. Indican is capable of being oxidized to form indigo blue. This oxidation is produced, in the usual test for indican in urine, by treating the latter with hydrochloric acid containing a little ferric chloride as oxidizing agent. The resulting indigo is removed from the reacting mixture by shaking it out with chloroform which dissolves the dye and becomes blue. The intensity of this blue color can be used as the basis for estimation of the amount of indican in the urine. Along with indican, the urine contains skatoxyl-sulphuric acid, phenol-sulphuric and cresol-sulphuric acid as other ethereal sulphates. The detection of these substances, all of which occur normally in very minute amounts, is more difficult than is that of indican. The usual method for estimating their amount in the urine is to precipitate sulphates by addition of barium chloride after the urine has been boiled with hydrochloric acid in order to liberate sulphuric acid from the ethereal sulphates. The resulting precipitate of barium sulphate is more than that obtained by treatment of unboiled urine with barium chloride. The latter precipitate of barium sulphate represents the inorganic sulphates. The difference between the amount of sulphates obtained by the method first described and the inorganic sulphates is a measure of the ethereal sulphates.

The origin of the ethereal sulphates through intestinal putrefaction of amino-acids was discussed in connection with bacterial action in the intestine (p. 493). The total ethereal sulphates of the urine have been shown to be not always parallel to the amount of protein putrefaction in the intestine. This indicates that they may originate through some process of the true metabolism of the body itself. But indican in the urine appears to be always an index of the extent of intestinal putrefaction. It thus becomes a valuable diagnostic sign. The conversion of indole and similar putrefaction products into sulphate compounds occurs in the liver, to which they are brought from the intestine by the portal blood.

Hippuric Acid.—The formula of hippuric acid represents it as benzoyl-amino-acetic acid. This means that it is formed by the union of benzoic acid and glycocoll.



Benzoic acid,



benzoates. It is also produced, in all probability, by the action of bacteria in the intestine. The animal body appears to be unable to oxidize benzoic acid but is able to convert this substance into the much less toxic compound, hippuric acid. This conversion is accomplished in the kidney and possibly in other organs. The production of hippuric acid involves synthesis in which benzoic acid is joined to glycocoll with elimination of one molecule of water. Benzoic acid and benzoates, when fed to man or animals, are excreted in the urine in the form of hippuric acid. The body is able to carry on this synthesis even when large amounts of benzoic acid are consumed. This affords protection against the toxic action of benzoic acid.

The amount of hippuric acid, though commonly small in human urine, is so large in that of herbivorous animals that hippuric acid is more abundant than any other constituent of their urine except urea. This is due to the comparatively large amounts of benzoic acid derived from vegetable foods. The name hippuric acid, was chosen because this substance can be crystallized readily from horse's urine. Hippuric acid forms long prismatic crystals when any urine, containing a large amount of it, is acidified with hydrochloric acid. This indicates that hippuric acid is present in urine, for the most part, as salts from which the free substance is liberated by acids.

The Skin as an Excretory Organ.—Although of less significance than the kidney, the skin also serves as an excretory organ. The sweat glands are its chief excretory structures. In man, they are tubular glands which are usually coiled so that the secreting surface is thereby increased. They consist of a single layer of epithelial cells. Their blood supply is maintained by the capillaries of the skin. Their activity is controlled, in part at least, by the sympathetic nerves. The part of the gland which is near the surface of the skin and serves as a duct is provided with non-striated muscle which probably assists in expulsion of sweat. The number of glands varies greatly in different regions of the human skin. They are most abundant on the palms of the hand, the soles of the feet and in the axillæ. The footpads of dogs and cats are the only regions provided with actively secreting sweat glands in these species. Some animals possess no sweat glands and are correspondingly less adaptable in their power to regulate body temperature (p. 619). The total number of sweat glands in man has been estimated to be about two million.

The skin also possesses sebaceous glands. These are located, with minor exceptions, close to the roots of the hairs upon which they pour out an oily secretion. This serves to decrease brittleness of the hair, thus aiding to preserve it. The sebaceous secretion also spreads over the skin. The cholesterol compounds of these secretions were described above (p. 72).

The skin is constantly sloughing off the outer epithelial cells which are replaced by the outpushing growth of deeper ones. This shedding process represents only a small loss of body material in the case of most animals. This process is comparable to the periodic molts that occur in certain reptiles and many invertebrates.

The Composition of Sweat.—Sweat is composed mostly of water, of which it normally contains about 99 per cent. The chief solid constituent is sodium chloride. Other salts are present in small amounts. These include phosphates, sulphates and lactates in traces. Urea is present in very low concentration (about 0.08 per cent). Small as this concentration is, the urea of sweat represents a not inconsiderable proportion of the total urea excreted. This is due to the large amounts of sweat that are secreted. Even in mild weather and without especial exercise an average adult man produces, according to some estimates, about 600 cc. of sweat per day. Under these circumstances, approximately 1.5 per cent of the total urea produced in the body would be excreted through the skin. In the case of profuse sweating, during muscular exertion in hot weather, as much as 3,000 cc. or more may be produced per day. This would carry nearly 10 per cent of the urea excretion. In addition to urea, uric acid, creatinine, ammonium salts and ethereal sulphates have been reported to be present in human sweat.

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CHAPTER XXIII

PROTEIN METABOLISM

So far as is known, proteins are produced exclusively by living things. Moreover, changes which appear to involve proteins are the constant accompaniment of all living activities. On this account, Verworn has suggested that living matter might be defined as the material within which protein metabolism occurs. This definition at least serves to call attention to the deep significance of protein metabolism in physiology. As explained above, protein synthesis occurs in plants. Very little is known concerning the nature of this process nor concerning the extent to which plants other than unicellular ones catabolize proteins. Protein metabolism has been studied chiefly in animals. The following discussion will deal for the most part with protein metabolism in mammals.

Proteins, derived either directly or indirectly from plant sources, are changed in the digestive system so as to liberate amino-acids. These are absorbed, for the most part, into the blood and are taken up by tissue cells in all parts of the body. Some of the amino-acids undergo deamination in the walls of the intestine and thus lead to the production of urea in the liver. But most of the amino-acids absorbed from the intestines are widely distributed in the body. Some of them are used for the production of the proteins of blood, the so-called circulating protein. Others are used for the synthesis of proteins in tissue cells, the so-called morphotic protein. Van Slyke has shown, however, that a large proportion of the amino-acids, taken into the blood from the intestine, are absorbed by tissue cells without being synthesized into proteins. They can be obtained from the tissues of an animal killed after a protein-rich meal by merely extracting with warm water. They are thus shown to be in a "mobile" condition; yet they are not free in the same sense that they are in blood plasma. As they do not diffuse out of the cells, they must be held in some non-diffusible but easily dissociable combination. This mobile quota of amino-acids in the cells is in contrast to the amino-acids which have been actually synthesized into the proteins of protoplasm. But even this, the morphotic protein, must be capable of undergoing catabolic changes. This is shown by the fact that the end products of protein metabolism continue to appear in the urine during starvation or during the use of a protein-free diet.

Exogenous and Endogenous Protein Metabolism.—The metabolism which involves the breaking down of amino-acids and of other substances which are derived directly from the food and which do not appear to

have been actually synthesized into the true morphological components of cells is called exogenous metabolism. Metabolism which involves the breaking down of actual tissue substance, the so called "wear and tear" of the body itself, is called endogenous metabolism. Although the distinction between these two types of processes cannot always be drawn sharply; yet where the distinction can be made, it is very helpful to an understanding of metabolism. In the case of metabolism of simple proteins, its exogenous aspect is really the metabolism of amino-acids. They are presumably absorbed and destroyed by the cells without ever having become a part of their indispensable vital structure. This exogenous metabolism varies greatly in extent, for it is determined solely by the amount of amino-acids supplied to the cells. Endogenous metabolism, on the other hand, is a comparatively uniform process, in that the cell proteins appear to break down continuously and inevitably, irrespective of the available amino-acid supply. For this reason, if for no other, amino-acids must be continuously furnished, along with the other foods required for cellular synthesis, in order to repair the ravages of endogenous catabolism.

Nitrogen Equilibrium. Non-storage of Protein.—Under normal circumstances, an adult animal excretes daily the same amount of nitrogen that is taken in with the food. Nitrogen intake represents protein almost exclusively; while nitrogen of the excreta represents the end products of protein metabolism and very little else. This equality of the nitrogen intake and output is called nitrogen equilibrium. In an animal observed over a short period (one or two days) nitrogen equilibrium may not be apparent, particularly when the protein content of the food fluctuates in amount. But, when the nitrogen balance for the animal is taken over a longer period, nitrogen equilibrium can be recognized, under all normal conditions, in the well-nourished adult animal. Excretion of nitrogenous waste products lags somewhat behind protein ingestion. This fact explains many cases of failure to recognize nitrogen equilibrium. Its recognition also depends upon complete collection of all excreta, especially of the urine and, in some cases, of the sweat. It also involves careful measurements of the food intake and analyses of both the food and the excreta.

Nitrogen equilibrium does not occur in the growing animal. Under these conditions, the formation of new morphotic protein causes the retention of amino-acids. This gives what is called a plus nitrogen balance. The same conditions prevail in an adult during the recovery from a tissue-wasting disease, such as typhoid fever, and during recovery from the effects of starvation or from those of a diet defective in the quantity or quality of its proteins. Conversely, a minus nitrogen balance (more nitrogen excreted than is consumed) occurs during the ravages of disease or of subnutrition.

The general tendency of the adult body to maintain nitrogen equilibrium is an index of the fact that the body lacks the power to store proteins in the same sense that it stores carbohydrates and fats. Glycogen and fat, especially fat, in most vertebrates and glycogen in many invertebrates, are stored in amounts that seem to be limited only by the amount of food available. The failure to store protein is therefore in marked contrast to the ability to store other foods. The only proteins stored are those used in structural growth and tissue repair and the only amino-acids stored are those which are held in small amounts as a "mobile" supply in the tissues. This amino-acid supply is probably subject to some fluctuation. This is indicated by the fact that the excretion of urea and similar nitrogen wastes tends to lag behind the ingestion of increasing amounts of protein food. But on any given diet the excretion of nitrogen wastes keeps pace, in the long run, with the protein ingestion. This means that protein food must be eaten by an animal almost daily and that no diet deficient in proteins can maintain an animal in health for more than a very limited period. This raises the question of how much protein is required to supply the minimum needs of the animal for maintenance of its endogenous protein metabolism. One way to study this question is to observe the nitrogen excretion during starvation. Under these circumstances, the body is using its own proteins, for it quickly uses up its "mobile" supply of amino-acids and must then draw upon the morphotic protein in order to maintain metabolism.

Protein Metabolism in Starvation.—Observations of the effects of starvation have been made chiefly upon men and dogs. Human fasts have covered from 30 to 50 days. Dogs often die from starvation after 30 to 40 days, though occasionally they show ability to withstand a longer fast. Fasting for 117 days with subsequent recovery was observed by Hawk in one experiment with a dog. In experiments to test protein metabolism, water must be given in adequate amounts in order to maintain satisfactory secretion of urine. In human beings, a complete fast maintained with water drinking, so as to avoid the discomfort or agony of thirst, is not painful except during the first few days. During this time the so-called hunger pangs are produced by violent muscular contractions of the stomach, but these cease after a time, usually about two days, and subsequent fasting is without depressing effects other than a tendency to muscular weakness and a general inactivity of the nervous and muscular systems. The composition of the urine during the time of fasting is typified by the results of the experiment shown in the table on page 653.

These figures show that, during the first day of starvation, excretion of the products of protein catabolism is not as large as it is later on. This is due to the fact that a store of glycogen present in the body is utilized during the first day so as to partially spare the body proteins. On a later

RESULTS OF URINE ANALYSES DURING FASTING

(From an Experiment Reported by Cathcart)

In 24-hour urine	First day of fast	Second day of fast	Twelfth day of fast	Fourteenth day of fast
Total nitrogen.....	10.51 g.	13.72 g.	8.77 g.	7.78 g.
Urea.....	19.2 g.	26.2 g.	14.2 g.	12.6 g.
Urea nitrogen.....	8.96 g.	12.26 g.	6.62 g.	5.99 g.
Urea N				
Total N × 100.....	85.0 per cent	89.0 per cent	75.0 per cent	77.0 per cent
Ammonia N.....	0.40 g.	0.73 g.	1.05 g.	0.73 g.
Ammonia N				
Total N × 100....	3.8 per cent	5.3 per cent	11.9 per cent	9.3 per cent
Uric acid.....	0.36 g.	0.18 g.	0.51 g.	0.51 g.
Creatinine.....	1.13 g.	0.91 g.	0.80 g.	0.64 g.
Creatine.....	0.06 g.	0.28 g.	0.28 g.	0.31 g.
Total sulphur.....	0.61 g.	0.80 g.	0.58 g.	0.54 g.
Total phosphates.....	2.26 g.	2.98 g.	1.55 g.	1.25 g.
Chlorine.....	3.29 g.	1.5 g.	0.18 g.	0.24 g.
Sodium.....		0.86 g.	0.09 g.
Potassium.....		1.33 g.	0.51 g.

date (third day of starvation) the body makes a larger use of its protein as is shown by increased nitrogen excretion. At this time the glycogen has been exhausted. Later on in starvation, nitrogen excretion reaches a lower level which is maintained with comparative uniformity. This is the starvation level and represents the minimum protein catabolism for fasting. It is known to be maintained until the approach of death from starvation. A few days before the fatal coma sets in, a marked rise in nitrogen excretion in the urine is observed. This is called the premortal rise in protein destruction. It marks the failure of the body to resist the destruction of proteins that are necessary for its life. The tissue proteins that are used in starvation up to the premortal stage are chiefly those of muscles. The starvation wastage of muscles is apparent even to casual observation. Their disintegration has been proved to occur by analyses of the tissues of animals killed after starvation. In addition to those of the muscles, those of the blood and possibly others may be used. The body shows the power to retain the composition of the most vital organs. The heart, the nervous system and the reproductive organs appear to withstand starvation without loss of their tissue proteins throughout long periods of fasting. These are the organs that are indispensable for the preservation of the individual and of the species.

During the progress of starvation, urea excretion decreases both relatively and absolutely. Relatively, it drops from an amount containing

85 to 89 per cent of the total nitrogen to an amount containing about 75 per cent of the total nitrogen. This signifies that urea does not predominate among endogenous protein waste products to as great an extent as it does among the exogenous ones. In some cases, urea may even decrease, so that it contains less than 50 per cent of the nitrogen of the waste products. Ammonia rises both in actual amount and in relation to the total nitrogen. This is due to the use of ammonia by the body, to prevent acidosis from the acids, which arise during defective oxidation in the absence of carbohydrate (other than that produced from protein). The rise of ammonia compensates largely for the decrease in urea.

Uric acid fluctuates during the first part of the fast but becomes very nearly constant later on. It is then an index of the starvation catabolism of nucleoproteins.

Creatinine decreases slightly but progressively. This is doubtless an indication of the decline of muscular efficiency. Corresponding to the decrease in creatinine, the creatine of starvation appears in the urine. This is an index of abnormality of protein metabolism in the adult.

The inorganic urine constituents give some additional information. The sulphates and phosphates run parallel to the excretion of total nitrogen. This indicates that they come from the catabolism of body proteins as do the nitrogen wastes. The fact that potassium is excreted in excess of sodium indicates that cellular constituents are being broken down. This is the case because the ratio of potassium to sodium is very much larger in cells than it is in the blood. Sodium and chlorine, though very much less abundant than they are in normal urine, continue to be excreted in small amounts throughout starvation. This shows the inability of the body to retain its NaCl completely.

The Minimum Protein Requirement.—Starvation does not give a true index of the amount of protein actually required to replace that which takes part in endogenous metabolism. This is explained by the fact that the starving body is obliged to use protein for oxidative metabolism to develop energy that would ordinarily be obtained by the use of carbohydrate. The limited transformation of protein into carbohydrate was explained above (p. 611). A truer measure of the amount of protein required to replace those which break down in normal "wear and tear" can be obtained in another way. This consists in feeding a diet which contains all body requirements except proteins. Such an experiment cannot be continued very long without disastrous results. An animal will die on such a diet almost as soon as though it were completely starved. But during brief experiments of this sort, such a diet is able to check the use of body protein for purposes of energy production and in this way gives some indication of the minimal endogenous protein metabolism. The results of such experiments show that the actual protein minimum, that is, the unavoidable breakdown of body protein is really very small. In

man, it is an amount of protein represented by an excretion of 2 to 3 grams of nitrogen per day from an adult of average size. This is calculated to represent about $\frac{1}{1,000}$ of the total protein in the body. With laboratory animals, correspondingly low figures have been established. For a pig of about 150 lb., endogenous protein metabolism may be so small as to result in the excretion of only about 3 gm. of nitrogen per day.

Nitrogen equilibrium can be used as an index of the adequacy of protein feeding. For this purpose it is merely necessary to find the smallest amount of protein which, incorporated in a diet that is adequate in non-protein constituents, serves to maintain nitrogen equilibrium. The quality of the proteins is important, but a mixture of proteins from suitably varied sources insures adequate quality. The diet must also furnish sufficient fuel for the calorific needs of the body. It must supply enough of the calories in the form of carbohydrate to maintain the ketogenic-antiketogenic balance. It must also furnish an adequate supply of inorganic salts, water and vitamins. A varied diet chosen from a combination of vegetable and animal sources can be made to fulfill all of these requirements and yet provide any desired degree of richness or poverty in protein. By the use of such diets, numerous investigators have studied the minimum protein requirements for man and laboratory animals. A notable series of such experiments was conducted by Chittenden. The subjects of these experiments included men of different habits of life: Professional men, athletes, office workers and soldiers. The diet in every case was cut down to a low level of protein intake without loss of nitrogen equilibrium. The amount of protein used varied from 35 to 50 gm. per day, according to the weight of the men. This amount of protein is contained in 175 to 250 gm. of lean meat or in 500 to 700 gm. of wheat bread. This represents less than half a pound of lean meat or less than one average sized loaf of bread. In most of the experiments, the actual nitrogen intake was between 5.5 and 7 gm. per day. Hindhede has reported an experiment upon a man who subsisted 178 days upon potatoes, onions and a small amount of fatty foods, giving a total nitrogen intake averaging about 4.4 gm. per day. The man was of average weight, 70 kilos (154 lbs.). During a large part of the experiment, he was in nitrogen equilibrium. For the entire period, he showed a small negative nitrogen balance, but appeared to be in good physical condition and well nourished at the end of the experiment. During a part of the time, he was in equilibrium on a nitrogen intake of 3.5 gm. per day.

The general conclusion from experiments of this type is that nitrogen equilibrium can be maintained by means of a protein intake surprisingly low in comparison with the amount of protein normally consumed by human beings. In most parts of the world, human adults consume,

according to statistical averages, more than 100 gm. of protein per day. (15-17 gm. of nitrogen.) But the minimum protein requirement or, more strictly speaking, the protein required to maintain endogenous metabolism, is evidently much lower than the usual intake. This conclusion does not give a decisive answer to the question of how much protein is best in the long run. This question will be discussed below.

The Specific Dynamic Action of Proteins.—This phrase is used to refer to the general fact that protein foods tend to speed up the oxidative metabolism of the body. In this respect, proteins stand in contrast to fats and carbohydrates, for when these substances are fed in excess of immediate needs, the extent to which they are utilized by the body is determined chiefly by the amount of muscular exercise and heat production. But when proteins are fed, they are largely oxidized, irrespective of the energy demands of the body. This is proved by measurements of the O₂ consumption and CO₂ production or by measurement of the heat production. Such observations are in agreement with the facts of nitrogen equilibrium and serve to indicate the inability of the animal organism to store up proteins. The oxidation of protein is accompanied, however, by the simultaneous oxidation of a certain amount of carbohydrate so long as the latter is available in the body for use. This fact has been established by the study of the respiratory quotients obtained during high protein feeding. Some of the ingested protein is itself converted to carbohydrate which may be oxidized along with protein. If carbohydrate is available from other sources, high protein feeding results in the storage of glycogen produced from the protein food. Very high protein ingestion, in an animal supplied with carbohydrate or containing glycogen in its tissues, may even result in the transformation of the carbohydrate, produced from the protein, into fat. But irrespective of utilization or storage of either carbohydrate or fat, the protein is bound to be largely oxidized in the animal whether it is at work or at rest. This forced oxidation does not change the temperature of the animal but merely increases heat loss. In the dog, increased lung ventilation, produced by heightened CO₂ production, has been shown to get rid of the extra heat formed in the body during high protein feeding.

The specific dynamic action of protein is not strictly characteristic of this kind of food alone. Carbohydrates and fats also exert a specific dynamic action in that they too may raise the rate of oxidative metabolism by their mere presence in the body. But they do not act as powerfully in this respect as protein does and they do not act in this way unless present in excess in the body. Protein foods, on the other hand, always exert a specific dynamic effect.

Protein-sparing Action.—In starvation, or during the use of a diet deficient in protein, the body makes use of its tissue protein. This is shown by a minus nitrogen balance. But if, under these circumstances,

non-protein food is eaten, the loss of nitrogen is diminished, that is, the body protein is spared. Carbohydrate is especially effective in this respect. Equal quantities of protein and carbohydrate are approximately isodynamic. In other words, a given weight of carbohydrate will replace an equal weight of protein for purposes of energy liberation in the body. But even aside from this advantage, carbohydrate appears to possess an especially high value. It is found that an animal can be kept in nitrogen equilibrium on a diet containing proteins and carbohydrates at a lower level of protein ingestion than is required for equilibrium on a diet of protein and fat. Thus carbohydrate spares body protein better than fat does. This is easily explained in view of the established fact that satisfactory fat oxidation requires carbohydrate, so that, in the absence of carbohydrate, body proteins would be destroyed in order to obtain the sugar necessary for fat utilization. Nevertheless, fats as well as carbohydrates exert some protein-sparing action. This is shown by the fact that the nitrogen elimination during starvation can be diminished by fatty foods.

The sparing of body protein is normally effected by the use of a mixed diet containing protein as well as other foods. This cuts down the destruction of body protein to the irreducible level of endogenous "wear and tear" metabolism and even this is continually replaced by anabolic processes, at least so long as nitrogen equilibrium is maintained. It is also possible to attain this same perfection of sparing of body protein by the use of protein alone, that is, by a diet containing only the very small amounts of carbohydrate and fat unavoidably present in protein-rich foods. Nitrogen equilibrium can be observed on such a diet. This result can be obtained even in an animal that has been starved until it is practically glycogen-and-fat free. In other words, protein can be used to supply the needs for both endogenous and exogenous metabolism—to replace "wear and tear" and to yield the requisite energy. This raises the question as to whether all proteins are equally effective in supplying nutritive needs. Consultation of the table on p. 30 shows that some proteins are distinctly deficient in their quota of amino-acids. Gelatin, for example, is practically devoid of tyrosine, typtophane and cystine. Gelatin does not maintain an animal or a man in nitrogen equilibrium. Its continued use, as the sole or chief protein of the diet is disastrous or even fatal. It is a nutritively deficient protein because it does not furnish all of the amino-acids required for construction of body protein. Yet gelatin has a sparing action in a certain sense. In starvation or during the use of a diet deficient in carbohydrate, gelatin can be used to check or even to prevent the breakdown of body protein. Gelatin is used for energy production in the body and doubtless is converted in part into sugar. If the nitrogen excretion of a starving man or animal is determined and the same subject is then given a protein diet

containing $\frac{1}{3}$ of the starvation nitrogen in the form of proteins, which yield an adequate quota of amino-acids, and $\frac{2}{3}$ in the form of gelatin, nitrogen equilibrium is reached. But when this diet contains $\frac{1}{4}$ "adequate" protein and $\frac{3}{4}$ gelatin, the nitrogen balance is minus. This means that about $\frac{1}{3}$ of the protein metabolized during starvation can be spared only by a supply of all of the amino-acids that are needed to repair the effects of endogenous metabolism; but about $\frac{2}{3}$ of the starvation breakdown of body proteins can be spared by the "inadequate" combination of amino-acids yielded by gelatin.

Certain ammonium salts have been found to exert a protein-sparing action similar to that of gelatin. Ammonium citrate, acetate and oxalate have been successfully used to cut down the loss of body protein during the feeding of experimental diets deficient in protein. Such results have been regarded, probably erroneously, as proof of the synthesis of proteins in the animal from ammonium salts. What it probably indicates is that ammonium salts can be used in the production of simple amino-acids, such as alanine and glycine, which the animal can use as a source of sugar, thus being spared the necessity of breaking down as much of its own body protein as it would otherwise require for energy production. This result can also be interpreted as indicating that an excess of ammonium salts in the body checks the deamination of amino-acids by mere mass action and thus enables the body to use its amino-acids as replacement material rather than using them for oxidation as rapidly as they are liberated in the body.

One possible explanation of this apparent usefulness of ammonium salts in animals must not be overlooked. This is the possibility that intestinal bacteria may synthesize proteins from ammonium salts and thus furnish the animal with useful protein. Such a synthesis is known to occur in both bacteria and yeast as well as in typical plants. Proteins so formed can be used by animals. Rats, for example, have been maintained on food which contained masses of bacteria as the source of protein. At most, the usefulness of ammonium salts for the synthesis and sparing of body protein must be very limited.

The Metabolism of Nucleoproteins.—Nucleoproteins are found in all cells. As they are particularly abundant in the nucleus, the name nucleoprotein was chosen. In view of this widespread occurrence, their presence in the food of animals is usually assured. White of egg, cheese, milk, highly refined flour and refined carbohydrate foods are among the few foodstuffs that do not contain nucleoprotein. The fact that an animal can grow on a milk diet indicates, as explained above, that an animal can synthesize nucleoproteins from materials not yet identified. At the same time, the animal is constantly catabolizing its nucleoproteins. This is shown by the presence of uric acid, purine bases and allantoin in the urine. The use of food nucleoproteins and the constant breakdown

of body nucleoproteins shows that these substances are concerned in both exogenous and endogenous metabolism.

They are compound proteins, formed by the union of nucleic acid and a simple protein. The character of the latter varies in the case of different nucleoproteins but its metabolism does not appear to differ from that of the simple proteins in general. On this account, the following discussion will be confined to the metabolism of nucleic acid, the distinctive part of the nucleoprotein molecule. Nucleic acid is liberated from nucleoprotein *in vitro* by the action of dilute mineral acids at room temperature. It is similarly liberated during digestion in the stomach. A corresponding liberation may occur in the body cells as a part of the endogenous breakdown of their nucleoproteins; but this is not known to be the case and need not necessarily be assumed to be a part of the catabolism of nucleic acid. It could doubtless be broken down without being first liberated from the protein complex of which it appears to be a part in the living cell. As a preliminary to a description of the catabolism of nucleic acid, its structure must be explained.

The Hydrolysis of Nucleic Acid.—Analysis of the completely hydrolyzed product, obtained by boiling nucleic acid with mineral acid, shows that four phosphate groups are contained in the nucleic acid molecule. This procedure also reveals that four sugar groups are present. The sugar obtained from nucleic acids of animal origin (from thymus gland and from fish sperm) is a hexose not yet identified. Its hexose character has been established by the fact that it yields levulinic acid and formic acid upon suitable oxidation (p. 106). Nucleic acid of plant origin (from yeast and wheat embryo) yields a pentose which Levene has shown to be d-ribose (p. 99).

In addition to phosphoric acid and sugar, the products of hydrolysis of nucleic acids include nitrogenous bases. Four of these are built into the nucleic acid molecule. Two are the purine bases, guanine and adenine, two are pyrimidine bases (p. 640) of which animal nucleic acids contain thymine and cytosine, while plant nucleic acids contain uracil and cytosine. These facts are summarized thus:

HYDROLYSIS PRODUCTS OF NUCLEIC ACIDS

Of <i>thymus</i> nucleic acid	Of <i>yeast</i> nucleic acid
(Typical of animal nucleic acids and probably the only one occurring in the nuclei of animal cells)	(Typical of plant nucleic acids and probably the only one in plant cells)
Phosphoric acid.....	4 mols
Hexose (unidentified).....	4 mols
Guanine.....	1 mol
Adenine.....	1 mol
Thymine.....	1 mol
Cytosine.....	1 mol
Phosphoric acid.....	4 mols
Pentose (d-ribose).....	4 mols
Guanine.....	1 mol
Adenine.....	1 mol
Uracil.....	1 mol
Cytosine.....	1 mol

The Nucleotides.—Evidence to prove the structure of nucleic acids has been obtained in numerous investigations, notably those of Levene and of Jones and their co-workers. Large fragments of the nucleic acid molecule have been isolated in pure form. Certain of these are called nucleotides. A nucleotide is a compound of a phosphoric acid group, a sugar group and a basic group (either purine or pyrimidine). Four different nucleotides have been obtained from the products of mild decomposition of yeast nucleic acid. Also, four different nucleotides are supposed, from indirect evidence, to be obtainable from animal nucleic acids, although only one of them has been actually isolated. The different nucleotides and their components are given in the following table.

	Nucleotide	Constituent groups
Obtained from yeast nucleic acid	Adenine penta-nucleotide Guanine penta-nucleotide Uracil penta-nucleotide Cytosine penta-nucleotide Thymine hexa-nucleotide Cytosine hexa-nucleotide Adenine hexa-nucleotide Guanine hexa-nucleotide	Adenine-pentose-phosphoric acid Guanine-pentose-phosphoric acid Uracil-pentose-phosphoric acid Cytosine-pentose-phosphoric acid Thymine-hexose-phosphoric acid Cytosine-hexose-phosphoric acid Adenine-hexose-phosphoric acid Guanine-hexose-phosphoric acid
Not actually obtained		

The first four of this list have been actually obtained in pure form from yeast nucleic acid. The fifth one has been obtained from thymus nucleic acid. The last three have resisted attempts to prepare them, but their occurrence in animal nucleic acids is inferred from the products of complete hydrolysis of thymus nucleic acid.

It is known that the sugar and the base are not both joined to the phosphoric acid group because the nucleotides combine with two equivalents of bases, such as NaOH, thus indicating that the phosphoric acid has two replaceable hydrogens. Moreover phosphoric acid can be split off by hydrolysis or by means of enzymes, leaving the sugar and the base joined together in what are called nucleosides. These will be described presently. The purine bases are split off from the nucleotides by mild hydrolysis or by means of specific enzymes. These facts are interpreted as signifying that the nucleotides have a sugar group in the center of the molecule with phosphoric acid on one side and a base (purine or pyrimidine) on the other, as shown in the following scheme.

Nucleotide

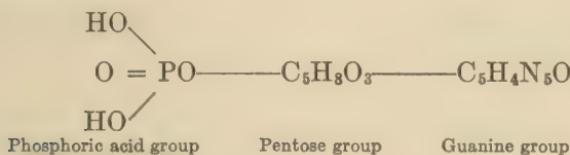
Sugar phosphate compound
(Freed from base by acid hydrolysis)

Base—sugar—phosphoric acid

Nucleoside

(Freed from the phosphoric
acid group by hydrolysis
in neutral solution)

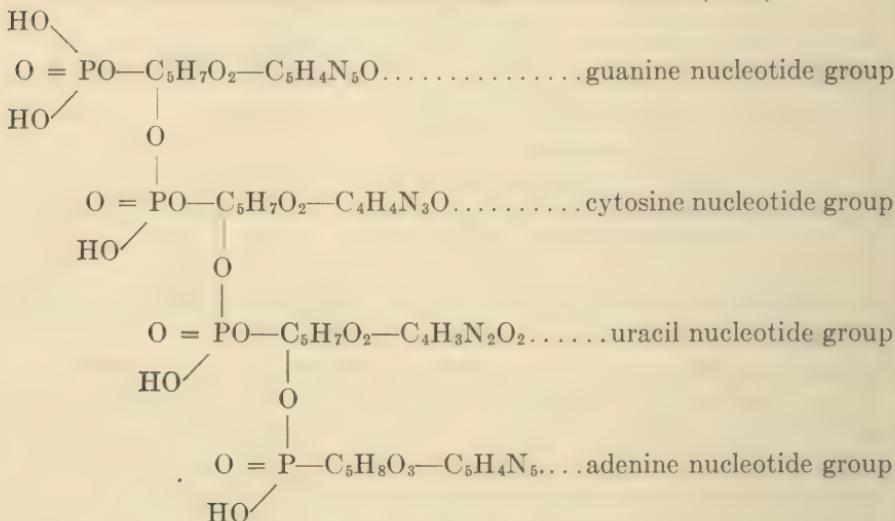
Two nucleotides have been found to occur, in very small amounts preformed in animal organs. These are guanylic acid of pancreas and inosinic acid of muscle. They stand in marked contrast, however, to the nucleotides that probably occur in animal nucleic acids. Both guanylic and inosinic acid contain the pentose sugar group (d-ribose), whereas the animal nucleic acids contain a hexose sugar group. This contrast suggests that these two acids are derived from plant foods of the animal. Guanylic acid appears to be identical in all its properties with the guanine penta-nucleotide obtained from yeast nucleic acid. Its structure is represented thus:



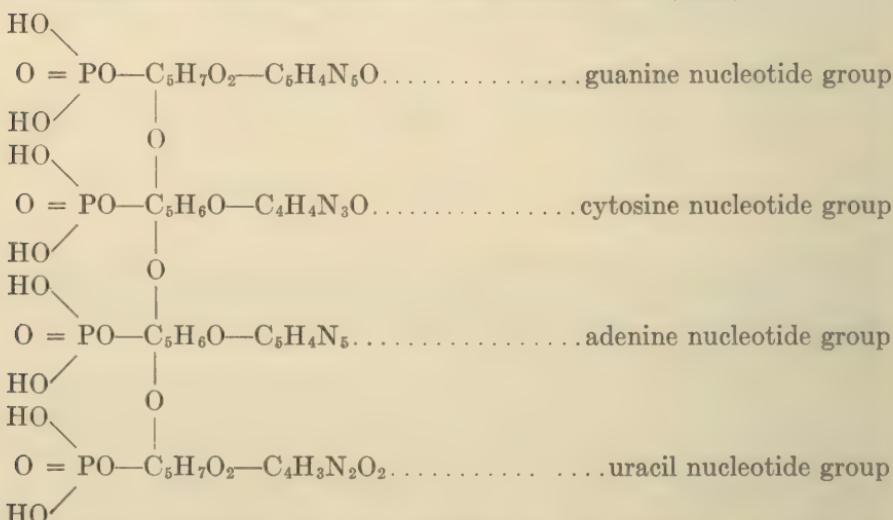
It breaks down, during hydrolysis with mineral acid, to form phosphoric acid, d-ribose and guanine as does the corresponding nucleotide, obtained from nucleic acid. Inosinic acid is similarly constructed but has hypoxanthine as the nitrogenous group.

The Structure of Nucleic Acid.—Nucleic acid itself is a tetra-nucleotide, that is, a union of four nucleotide groupings. The manner in which they are joined to each other is still in debate. In the case of yeast nucleic acid, two leading theories, that of Levene and that of Jones, have been advanced. Levene's theory assumes that the phosphoric acid groups of the nucleotides are used to effect unions. Jones believes that nucleotides are joined in nucleic acid by their sugar groups, forming an ether-like linking. He bases this theory partly upon his observation that the splitting of yeast nucleic acid into nucleotides, by the action of pancreas nuclease, does not increase the acidity of the mixture. This indicates that phosphoric acid groups could not have any of their combining powers used up in the joining of nucleotides in the nucleic acid molecule. The structural formulas proposed for yeast nucleic acid by Levene and Jones are represented thus:

Levene's Structural Formula for Yeast Nucleic Acid (1919)

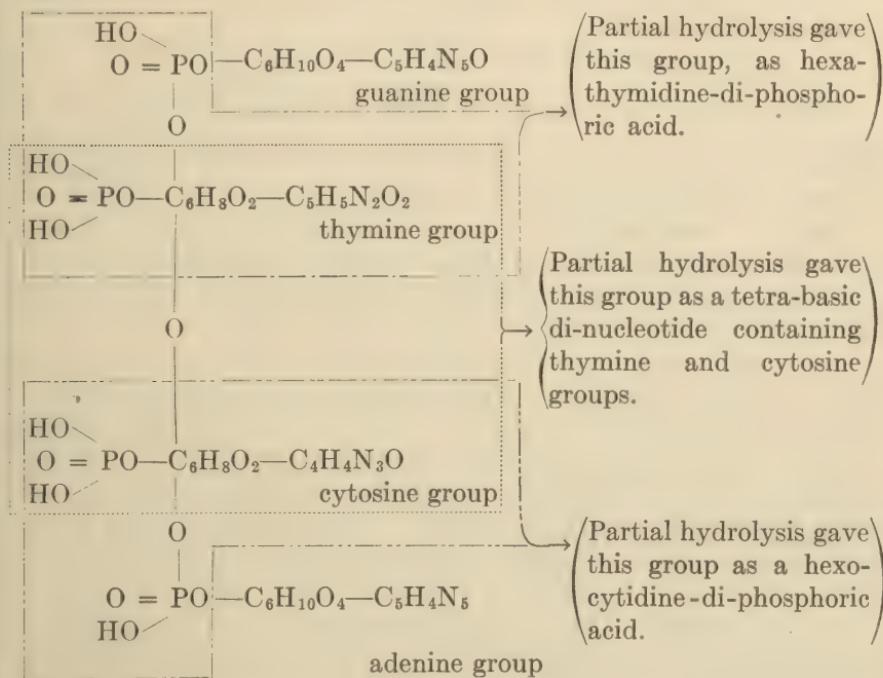


Jones's Structural Formula for Yeast Nucleic Acid (1920)



The formula proposed by Jones accounts for the fact that acidity does not increase when the nucleic acid is converted into four nucleotides. The possibility of union of the nucleotides through the basic groups appears to be eliminated by the observation of Jones that purine bases are set free from nucleic acid, during acid hydrolysis, just as rapidly as they are from nucleotides. The order of arrangement of the nucleotides is still in doubt and is based upon indirect and incomplete evidence. The manner of union of the sugar group with the phosphoric acid and with the basic group is also in need of further investigation.

Levene has also proposed a formula for animal nucleic acid. It is based partly upon the general theory of the tetra-nucleotide structure of nucleic acids and partly upon the isolation of what Levene believes to be three different, large fragments of nucleic acid. These were obtained by partial hydrolysis of fish sperm nucleic acid and appeared to be: (1) Hexo-thymidine-di-phosphoric acid, (2) a di-nucleotide, containing thymine and cytosine and having the combining powers of a tetra-basic acid, which shows that the two nucleotides are not joined by the phosphoric acid groups and (3) hexo-cytidine-di-phosphoric acid. The constitution of these three is indicated in the following structural formula, assigned by Levene and Jacobs to animal nucleic acid:

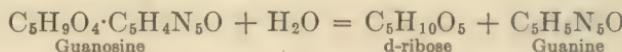


The hydrolysis was carried out in 2 per cent sulphuric acid. It apparently caused different decompositions of nucleic acid molecules so as to produce these different fragments. The purine nucleoside groups yielded free purine bases and levulinic acid.

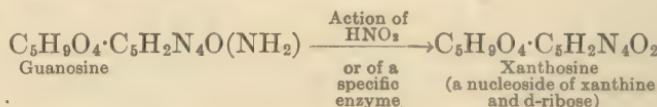
Nucleic acids of different origin may vary as to their structure; but, so far as they have been studied, they seem to fall into the two classes: Plant nucleic acids, typified by that of yeast, and animal nucleic acids typified by that of the thymus gland.

Nucleosides.—Compounds, consisting of one of the sugar groups and one of the basic groups of a nucleic acid molecule are called nucleosides. Examples are: Guanosine (pentose-guanine compound), adenosine (pen-

tose-adenine), cytidine (pentose-cytosine) and uridine (pentose-uracil). These four have been prepared in pure crystalline form from yeast and wheat nucleic acids after suitable hydrolytic decomposition with dilute ammonia. They may be called pentosides. Each of these is converted into its constituent base and sugar by hydrolysis with hot mineral acid. The reaction with guanosine, for example, is:



Guanosine is converted into another similar nucleoside, xanthosine, by deamination with nitrous acid or by the action of a specific deaminating enzyme. This reaction may be represented thus:



Both guanosine and xanthosine can be produced from guanylic acid. Adenosine (adenine pentoside) is similarly converted into inosine, the pentoside of hypoxanthine. Inosine can be obtained from inosinic acid and is found in muscle, so that it probably originates from inosinic acid in the body. Cytidine (cytosine pentoside) can be converted into uridine (uracil pentoside) by the action of nitrous acid.

The Catabolism of Nucleic Acid by Animal Enzymes.—The progress of the metabolic breakdown of nucleic acid has been studied, for the most part, by means of observations of the effects of animal tissue extracts upon yeast nucleic acid and upon the compounds obtained by its partial hydrolysis. Similar investigations with nucleic acids of animal origin have not been extensively conducted. But the catabolism of animal nucleic acid is probably similar to that of yeast nucleic acid. The process results in the formation of the same urinary end products, so far as they are known. When animal organs that are rich in nucleic acid, as for example, thymus gland and liver, are fed to men and animals, the uric acid or allantoin of the urine is increased just as it is after feeding nucleic acids of plant origin. Endogenous metabolism of the nucleic acids of the animal's own cells may differ, in some respects, from that of the exogenous metabolism of nucleic acids. Information on this point is still meager; but in any case, the final products appear to be much the same.

The following enzymes have been found to be concerned in the catabolism of nucleic acid:

1. Nucleinase—changes nucleic acid to nucleotides. There may be more than one of these enzymes. Nucleinase is found in intestinal juice, the intestinal mucosa, kidney, heart muscle, liver, pancreas and blood.

NUCLEOTIDASES

2. *Phospho-nucleases*—change nucleotides by hydrolysis so as to liberate phosphoric acid and nucleosides. There may be four different enzymes of this type, each specific in its effects upon a given nucleotide. They are found in the intestinal mucosa, kidney, heart muscle and liver.

3. *Purine-nucleases*—cause the hydrolysis of purine nucleotides so as to liberate the purine base. The fate of the sugar phosphoric acid part of the nucleotide has not been shown. There may be two different enzymes of this type, each specific in its action on a given purine-nucleotide. But in the case of these, as well as that of the phospho-nucleases, their number is not known. Purine-nuclease has been found in pancreas, but its occurrence in other organs is not well established.

DEAMINASES

These split off ammonia from free purine bases or from nucleosides and convert the amino-purine base into the corresponding oxy-purine derivative.

4. *Adenase*—converts adenine into hypoxanthine.
5. *Guanase*—converts guanine into xanthine.
6. *Adenosine-deaminase*—converts adenosine into inosine.
7. *Guanosine-deaminase*—converts guanosine into xanthosine.

NUCLEOSIDASES

These cause the hydrolysis of purine nucleosides to liberate the purine base and the sugar.

8. *Adenosine-hydrolase*—converts adenosine into adenine and sugar.
9. *Guanosine-hydrolase*—converts guanosine into guanine and sugar.
10. *Inosine-hydrolase*—converts inosine into hypoxanthine and sugar.
11. *Xanthosine-hydrolase*—converts xanthosine into xanthine and sugar.

Adenine and guanine, liberated by (8) and (9) respectively, may be further acted on by (4) and (5) so as to be changed to the corresponding oxypurines.

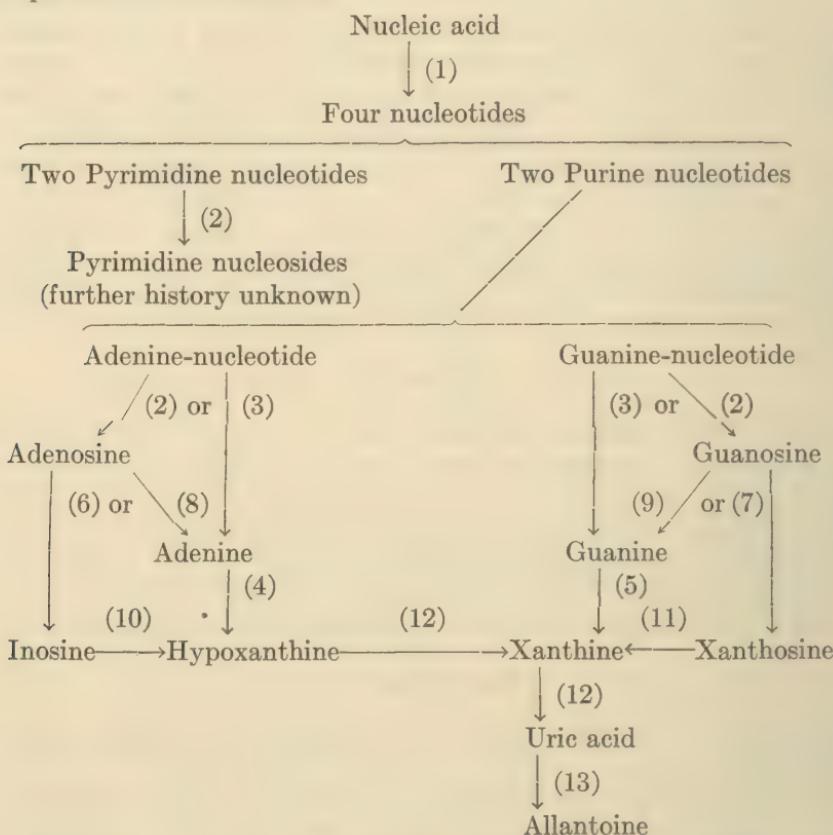
OXIDIZING ENZYMES

12. *Xanthine-oxidase*—oxidizes hypoxanthine to xanthine and xanthine to uric acid.

13. *Uricase*—oxidizes uric acid to allantoin.

The following scheme represents the various possibilities, so far as they are known, for the breakdown of nucleic acid in the animal body. The numbers, used in the scheme, correspond to those placed before the names of the enzymes in the above list. Each arrow indicates the metabolic reaction catalyzed by the enzyme whose number is beside the arrow. Compounds, other than purines and those containing purine groups, are

not indicated in the scheme because their fate is either that of similar compounds or is unknown.

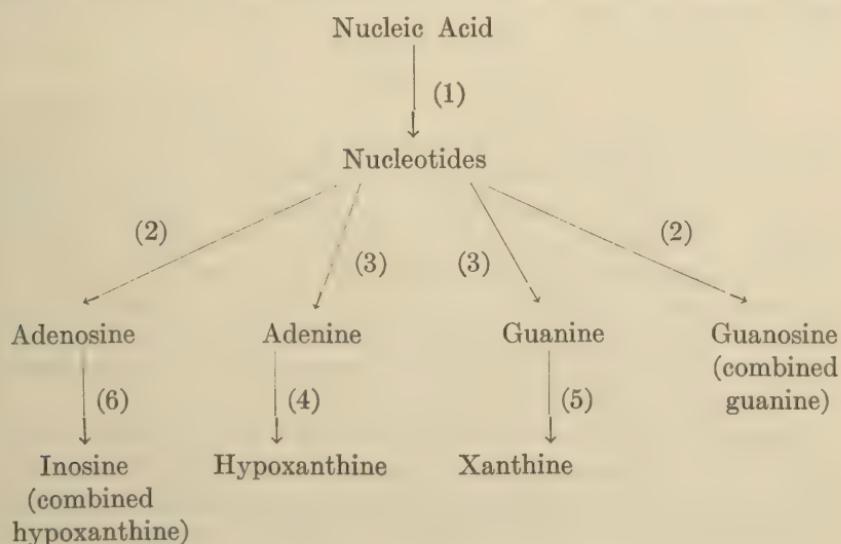


Enzymes 4 to 12 are variously distributed among the different organs of the body and are so differently distributed among the organs of different species that no very useful catalogue of their occurrence can be presented. In the case of every one of these enzymes, some organ has been found to contain it, as shown by the action of organ extracts, without containing any of the enzymes of similar action which might be confused with it. For example, Jones has shown that livers of the four animals, ox, pig, rabbit and dog, have a differing complement of the enzymes concerned with uric acid production. This is shown, schematically, as follows:

	Livers of			
	Ox	Pig	Rabbit	Dog
Adenase.....	+	+	0	0
Guanase.....	+		+	+
Xanthine-oxidase.....	+	+	+	0

The presence or the absence of these enzymes is shown by adding adenine or guanine or both to extracts of the liver and, after a suitable digestion period, analyzing for purine substances. In the case of the ox liver, uric acid is the chief end product together with some xanthine. If oxygen is plentifully supplied to the digestion, allantoin is the chief product. These results indicate the presence of adenase, guanase, xanthine-oxidase and uricase. In the case of the pig liver, guanine is not changed, while with rabbit liver adenine is unchanged; otherwise, results with these two kinds of liver are the same as with ox liver. In the case of the dog liver, adenine and xanthine but no hypoxanthine or uric acid can be recovered at the end of the experiment. This indicates that dog liver contains guanase but no adenase or xanthine-oxidase.

In order to determine what enzymes of this entire group are present in any given organ, Jones employs the following procedure. A water extract of the organ is allowed to act upon yeast nucleic acid in the presence of antiseptics. After a suitable time, a part of the material is subjected to analysis for determination of free phosphoric acid and free purine compounds. The rest of the material is subjected to hydrolysis with boiling sulphuric acid and is then similarly analyzed. The results of an experiment will illustrate the principle involved. With pancreas, the material, before acid hydrolysis, contains in free form all of the phosphoric acid of the nucleic acid and also contains xanthine and hypoxanthine. The material, after acid hydrolysis, contains more hypoxanthine than before, and, in addition, contains guanine. This proves that digestion produced free xanthine and hypoxanthine and combined guanine and hypoxanthine. Jones represents these results by the following scheme in which the numbers indicate the same enzymes which they represent on p. 665.



These results show that the pancreas contains 1. nucleinase, 2. phospho-nuclease, 3. purine-nuclease, 4. adenase, 5. guanase and 6. adenosine-deaminase. It appears to lack enzymes indicated by numbers 7., 8., 9., 10. and 12. of the scheme on p. 666.

Although the dozen or more enzymes that are known to be concerned with nucleic acid catabolism are not uniformly distributed in the body, yet among the organs of any animal there are enzymes sufficient to convert the nuclear purines to uric acid. In the case of the human body, adenase appears to be entirely lacking from all organs, yet adenine is converted to uric acid by way of inosine and hypoxanthine. In similar ways, other animals compensate for the lack of certain nuclear enzymes by the use of others. The lack of uricase in the human body has no compensation and exposes man to the chance of uric acid accumulation in the tissues.

Phosphorus Metabolism in Relation to Proteins.—The body maintains a nice balance between the intake and output of inorganic constituents. This adjustment has been particularly studied in connection with the phosphorus balance. Although the phosphate salts of the diet may furnish part of the ingested phosphorus especially when milk is taken, yet the phosphorus of food proteins must also be considered. Two types of proteins are important sources of phosphorus: The nucleo-proteins and the phospho-proteins. The latter include caseinogen of milk and vitellin of eggs. The phosphorus of nucleo-proteins of the food is eliminated in the urine as phosphate. The total phosphates of the urine may be more than doubled by the use of a diet rich in nucleo-protein. Caseinogen or casein and vitellin also contribute to the exogenous production of urinary phosphates. The processes by which phosphoric acid is liberated from these proteins have not been determined but probably involve some oxidation, for the phosphate groups of these proteins cannot be recognized *in vitro* until the protein has been oxidized.

The animal shows a tendency to remain in phosphorus equilibrium under normal conditions of adult life. But during growth and conditions of recovery a plus balance (retention of phosphorus) occurs. A large phosphorus retention results from bone formation during certain stages of growth. The disease of rickets is accompanied by a minus phosphorus balance owing to the failure of phosphorus retention in the bones. Under these conditions, inorganic phosphates and phosphorized proteins are both ineffective in correcting the tendency of the body to lose its phosphorus, although under ordinary conditions of health, both contribute equally well to the phosphorus needs of the animal.

These needs, like those for amino-acids and other animal nutrients, cannot be met by anything less than an irreducible minimum supply which is equivalent to the loss of phosphorus as a result of endogenous metabolism. The phosphorus loss has been observed during starvation

and is found to be roughly proportional to the protein catabolism as represented by nitrogen excretion. The ratio of the nitrogen of the urine to the total phosphates (computed as P_2O_5) is fairly constant during starvation. Variations between representative observations are:

$$\frac{N}{P_2O_5} = 4.2 \text{ to } 5.2$$

This indicates that the metabolism of starvation involves the breakdown of nucleoproteins along with that of other proteins. This indication is confirmed by the constant and uniform excretion of uric acid which is observed during starvation.

The fact that phosphates and phosphoproteins or even phosphates alone can supply all of the phosphorus needs of the growing animal indicates that the body can synthesize nucleoproteins and phospholipins from relatively simple constituents.

Sulphur Metabolism in Relation to Proteins.—A varying but always large proportion of the sulphur intake of the body is in the form of cystine of proteins. Protein metabolism involves the disposal of this sulphur. The small amount of glutathione in animal tissues could account for only a small part of it. The extent to which glutathione may be broken down in metabolism is unknown. For the most part, cystine is oxidized in the body so as to produce sulphate groups. Inasmuch as the average cystine content of the proteins of a mixed diet tends to be uniform, the excretion of sulphates in the urine tends to run parallel to that of the total nitrogen of the urine. During starvation, this parallelism is especially marked. The ratio of nitrogen to sulphur of the urine is between 14 to 1 and 16 to 1. This ratio derives interest from the fact that muscle tissue has about 14 times as much nitrogen as sulphur. Muscle proteins are known to be used up during starvation.

The Products of Endogenous and Exogenous Metabolism.—The breakdown of the body proteins and that of food proteins results, for the most part, in the same products. Ammonium salts, urea, uric acid, purine bases and allantoine are produced by both types of processes. Creatine and creatinine represent endogenous metabolism excepting in so far as they appear in the urine after they have been ingested as such. They are not produced by exogenous metabolism of proteins. This is shown by the fact that their amounts are not influenced by variations of protein intake. In man, allantoine is excreted in small amounts, probably as a result of its inclusion in the food. In the absence of uricase, no process of allantoine production appears to occur in the body. Ethereal sulphates, conjugated glycuronates and hippuric acid are partly exogenous and partly endogenous in that the aromatic groups of the ethereal sulphates and

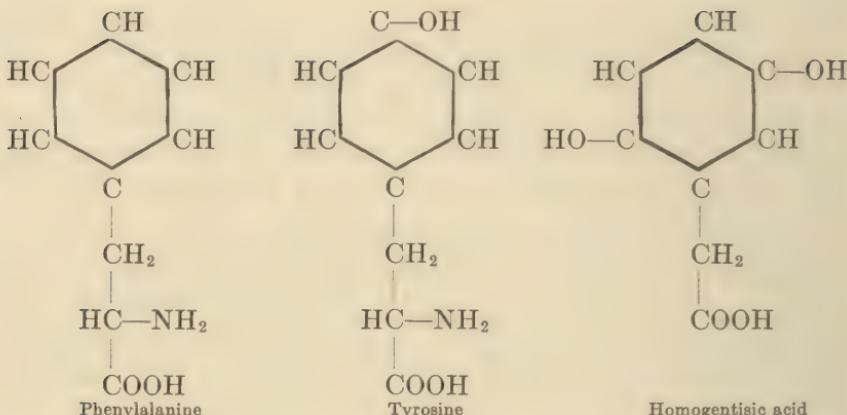
conjugated glycuronates and the benzoyl group of hippuric acid are derived directly from ingested material, while the sulphate and glycuronate groups and the glycine group of hippuric acid may be either exogenous or endogenous. The inorganic phosphates and sulphates of the urine are partly exogenous and partly endogenous. In the case of many of the excretory products which appear in very small quantities, a decision as to the exogenous or endogenous nature of their origin is difficult. An endogenous origin of a waste product is proved when it is found to be excreted during the use of a diet which is free from everything that might serve as its exogenous source.

Protein Metabolism in Relation to Muscular Work.—Normally, muscular work is done at the expense of carbohydrate or of carbohydrate and fat. But in the absence of sufficient carbohydrate supplies, protein can be utilized for this purpose. It yields a certain amount of glucose, or at least something which behaves like glucose in metabolic processes. A protein according to Lusk, may yield as much as 58 per cent of its weight as carbohydrate, when it is metabolized under certain conditions. This explains the possibility of doing muscular work during starvation or during the use of a diet that is deficient in carbohydrate. The amount of protein catabolized can be estimated from the urinary nitrogen excretion. It is not increased by muscular work during starvation, in case there is fat still available in the body. This suggests that the body protein is broken down to furnish a supply of cell nutrients, including carbohydrate, irrespective of whether the muscles are at rest or in action; so that when muscular work is done, the carbohydrate is already available to such a degree that protein catabolism does not need to be increased. Fat, if present in the starved organism, is oxidized more rapidly during muscular work than during rest. This is shown by measurements of CO_2 excretion and determinations of the respiratory quotient. When fat is not available, protein catabolism is increased by muscular work during starvation or during the use of a carbohydrate-free diet.

During conditions of good nutrition on an adequate mixed diet, muscular work has no effect on the total amount of protein catabolized during the day (p. 309). The individual nitrogenous waste products are none of them altered in amount as the result of any degree of muscular exertion, short of real exhaustion. If the protein intake is not altered, the amounts of urea, ammonia, creatinine and uric acid in the 24-hour output of urine are the same on a day of complete rest as they are on a day which includes 10 or 12 hours of muscular work. Several investigators have found, however, that the hourly excretion of creatinine and uric acid is influenced by muscular activity, being increased during the two or three hours which follow exercise. The output of both of these wastes tends to be greater during the daytime than it is during the period of rest at night. The periods of increased excretion of these substances

after muscular work are followed by periods of diminished excretion which so compensate for the working period that muscular activity appears to exert no effect upon the total day's output of creatinine and uric acid. On this account, the influence of muscular work upon their production cannot be considered as established. Possibly contraction merely favors the elimination of creatinine and uric acid from muscle rather than their actual production.

Certain Disturbances in Protein Metabolism.—Some abnormalities in catabolism of proteins are revealed by the presence in urine of unusual protein wastes. Although the explanation of their presence is not yet adequate, they derive interest from the fact that in some cases, they disclose possible intermediary products of normal protein metabolism. One of them is cystine. This amino-acid may appear in the urine in quantities sufficient to crystallize out in its characteristic six-sided plates. The condition is called **cystinuria**. It may persist for many years without being accompanied by any apparent ill health. It indicates that free cystine may arise in the body at a stage in protein metabolism. Another abnormality is the presence of various amino-acids in the urine in quantities far in excess of the normal trace. Of these only **tyrosine** has been identified by formation of characteristic crystals. Although such excretion of amino-acids appears to be the result of pathological conditions, such as cirrhosis of the liver, it helps to confirm the theory which postulates that protein metabolism involves free amino-acids. A third abnormality is the presence of plasma proteins in the urine. This condition is called **albuminuria**. It results from several types of kidney disease, but kidney disturbance is not always detectable as an accompaniment of this condition. If a kidney in its normal state can allow blood proteins to pass through it, they must become more diffusible than they normally are. Another kind of protein, which sometimes appears in urine, is called "**Bence-Jones protein**." It has the characteristics of certain proteoses and is the result of tissue destroying diseases, such as carcinoma and the bone disease called osteomalacia. Its elimination under these circumstances and the complete absence of proteoses and peptones from normal urine give some indications that protein metabolism does not normally pass through the hydrolytic stages of proteose and peptone. A rare abnormality in protein metabolism is marked by what is called **alcaptonuria**. The urine when treated with ammonia turns to a brownish green color which gradually darkens to black. This is due to the presence of large amounts of **homogentisic acid** which may be excreted to the extent of 10 gm. or more per day. Tyrosine and phenylalanine, when fed to persons with alcaptonuria, are converted almost quantitatively into homogentisic acid. The chemical relationship of these three substances is shown by their structural formulas.



Alcaptonuria has been interpreted as signifying that, in normal metabolism, phenylalanine and tyrosine pass through the stage of homogentisic acid, but in case of alcaptonuria, the body has lost its power to further oxidize the six-membered carbon ring. This idea is based on the observation that homogentisic acid, when fed to normal persons does not appear in the urine but is completely oxidized. It is excreted unchanged in alcaptonuria. Moreover, all three substances, phenylalanine, tyrosine and homogentisic acid, are converted into the same end product, aceto-acetic acid, when perfused through a normal excised liver. These observations do not necessarily prove the normal course of oxidation of these substances. If homogentisic acid is a normal intermediary product, the feeding of sufficiently large amounts of tyrosine should cause the excretion of some homogentisic acid in the urine of even a normal person. But this experiment generally fails. Abderhalden obtained a very small amount of homogentisic acid in the urine in one experiment in which 50 gm. of tyrosine were given to a normal man in one day. The course of oxidation of tyrosine (and all aromatic ring compounds) in the body requires more investigation.

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CHAPTER XXIV

THE AMOUNT OF FOOD REQUIRED BY ANIMALS

The nutritive requirements of plants were outlined in Chap. I; those of animals have been indicated qualitatively in several different connections in the preceding pages. The animal's constant need of protein or, more strictly speaking, the need for a supply of all the different amino-acids, was shown in Chap. II. The needs for inorganic salts were described in Chap. V. The vitamin requirements were explained in Chap. VIII. The need of carbohydrate food, or of something which can furnish carbohydrate in metabolism, was discussed in Chap. XXI. A diet must fulfill all these requirements if it is to nourish an animal successfully. It now remains to discuss the amount of food required. This is most commonly measured in terms of calories. Such energy units are particularly convenient because, in accordance with the law of conservation of energy, the total calorific needs of an organism can be determined by finding the total of its energy transformations.

The Animal Calorimeter.—The apparatus used for measurement of the energy transformations of an animal is a calorimeter. The modern type of animal calorimeter is exemplified by the Atwater-Rosa-Benedict form, designed for use in experiments on man. This is the most completely equipped type of calorimeter. It measures, simultaneously, the respiratory exchange and the heat production of the subject of the experiment. The main part of the apparatus is a chamber which can be hermetically sealed. Its size depends upon whether the subject is to lie still or to be permitted to move about during the experiment. Comparatively small calorimeters are constructed for experimentation upon animals.

For the purpose of measuring the respiratory exchange and to ventilate the air of the chamber, an inlet and an outlet are provided. A circulation of air is maintained through the chamber and a connecting system of closed tubes and containers (Fig. 169). A suitable air blower is included in the circuit to maintain air movement. The air enters the chamber after being dried and warmed. As it leaves the chamber, it is forced through sulphuric acid in order to remove the moisture evaporated from the subject. The acid is held in "absorbers" which can be weighed at the beginning and the end of the experiment. Their gain in weight shows the amount of water evaporated from the respiratory passages and the skin. After leaving the sulphuric acid, the air is also forced through

containers filled with soda lime, which absorbs CO₂. The water which evaporates from these containers is absorbed by the sulphuric acid between them and the air inlet of the chamber. By weighing these soda lime and sulphuric acid containers before and after the experiment, their combined increase in weight is measured. It represents the CO₂ pro-

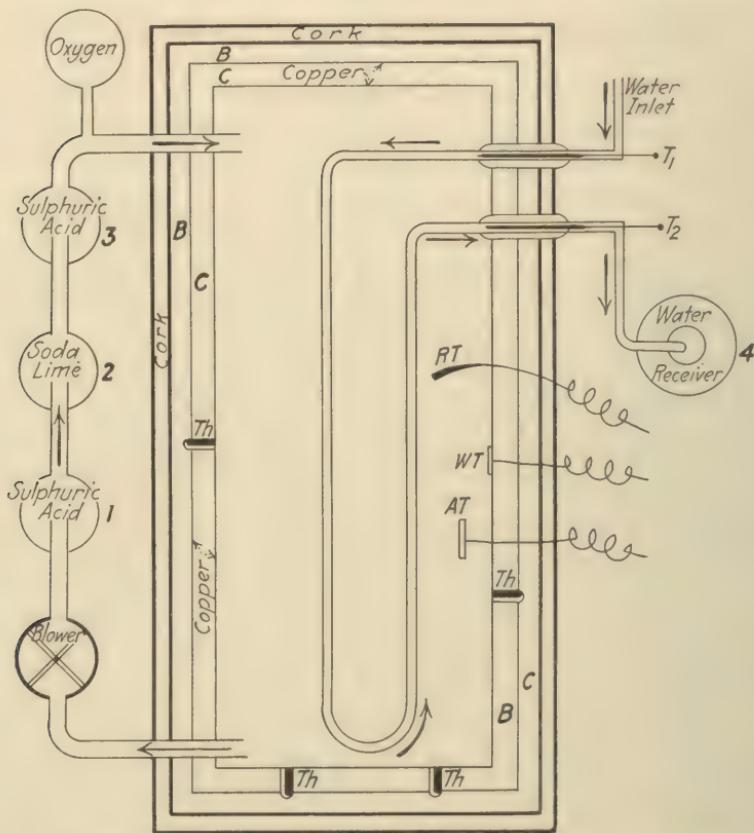


FIG. 169.—Diagrammatic plan of respiration calorimeter, Atwater-Rosa-Benedict type. Absorbers at 1, 2 and 3. B and C, dead air spaces. Th, thermocouple. T₁ and T₂, thermometers for water as it enters and leaves the chamber. RT, rectal thermometer. WT, wall thermometer. AT, air thermometer. (After Lusk).

duced by the subject of the experiment. Oxygen utilization, which tends to deplete the supply of it in the air circulating through the apparatus, is compensated for by additions of this gas from a tank of compressed oxygen. The latter is so arranged that the diminished pressure due to oxygen utilization automatically admits oxygen from the tank to replace that used. The loss of weight of the tank during the progress of the experiment gives the amount of oxygen used, provided due allowance is made for any change in the O₂ concentration of the system, for changes in its temperature and for barometric changes. The composition of the

air in the chamber is determined, analytically, from fair samples pumped out at the beginning and the end of the experiment. The temperature and pressure of the air in the chamber is also taken at these two times. By these various appliances, the apparatus permits the measurement of the H_2O and CO_2 given off and of the O_2 used during the time of observation. This is, in many cases, one hour, but in certain experiments the subject remains in the calorimeter during longer periods. Experiments lasting ten days are on record. In these cases, food is passed in through a porthole with inner and outer airtight doors. The excreta are similarly removed.

The calorimeter also permits the measurement of heat production. For this purpose, a flow of water is maintained through a coil of copper pipes suspended from the roof of the chamber. The water as it enters the coils is at a constant, controlled temperature, lower than that of the air of the chamber. Consequently, it absorbs the heat evolved by the occupant, acting in a way that is just the opposite of the action of an ordinary hot water heating system. The water which flows out of the coil is collected in a tank which is weighed at the beginning and the end of the experiment. Thus the amount of water which flows through is known. Electrical conductivity apparatus is attached to the water pipes at the points where they enter and leave the chamber. This permits frequent and very accurate recording of the water temperature at these points. From the data (volume of water passing through and average temperature at entrance and exit points) the heat evolved by the subject can be computed, with certain corrections described below, for any period of the experiment or for its entire duration.

Obviously, loss of heat, by radiation from the chamber, must be prevented. In experiments upon very small animals, this object is attained by the use of a Dewar flask with its surrounding vacuum which provides the heat insulation. But in a large calorimeter, such as is used for human experimentation, the vacuum insulation principle is not practicable. Instead, heat insulation is secured in part by the structure of the chamber. It has three walls separated by dead air spaces which entirely surround the chamber. The outermost of the three walls is especially constructed with cork interlining so as to afford heat insulation. The final attainment of heat insulation is reached by a device which depends upon the principle that heat does not radiate between bodies of the same temperature. The two inner walls are made of copper. An electrical thermocouple registers any minute temperature differences between them. An electrical heating system applied to the outer copper wall is regulated so as to keep the temperature of this wall the same as the inner one. Thus, heat loss through the walls of the chamber is effectively prevented and all heat given off by the occupant must be conveyed away from the chamber by the air and water currents. The air entering the chamber

is warmed to the temperature which prevails within so that it is not itself warmed by the body heat of the subject.

A large part of the heat produced by the body is used in evaporation of water from the respiratory passages and the skin. Because of the high latent heat of water, a quarter or more of all the heat produced by the body is taken up as latent heat during evaporation. The actual amount can be reckoned from the quantity of water which is removed by the outgoing air. This quantity is determined by weighing the water absorbers and, as the latent heat of water is a well-established constant, the heat removed by evaporated water is satisfactorily computed.

Certain other corrections must also be taken into account. These include the temperature of the air in the chamber at the beginning and end of the experiment and the body temperature of the subject at the beginning and end. Both of these temperatures are obtained by means of electrical resistance thermometers which can be read outside of the chamber. One thermometer is suspended in the air of the chamber, the other is inserted in the rectum of the subject. Inasmuch as the specific heats of air and of the animal body are approximately known, the readings of these thermometers permit the computation of any heat diminution or heat storage that may occur in the air or in the body of the subject during the experiment.

Direct and Indirect Calorimetry.—The calorimeter really permits of two distinct and different computations of the amount of energy transformed by the body. One of these is the result of the so-called direct calorimetry, that is, the actual measurement of heat loss. The other is the result of the so-called indirect calorimetry, that is, the computation of heat loss from the respiratory exchange. From the latter, the respiratory quotient is determined. At the same time, the urinary excretion of nitrogen is measured; so that the amount of protein catabolized can be computed. This, together with the respiratory quotient, and the figures for the total O₂ utilization furnish the data for computation of the total amount of each kind of nutrient (protein, fat and carbohydrate) oxidized in the body during the experiment. The amount of heat produced by such oxidations is known from bomb calorimeter measurements (p. 73). It is therefore possible to calculate the total calories of heat produced in the body although only these indirect measurements are used. Comparison of the figures obtained by direct and indirect calorimetry furnishes a check upon the accuracy of any given experiment. If the subject lies perfectly still, so that the only energy utilized for muscular contraction is that required for breathing movements, the heart beat and the contractions of involuntary muscles, all of which can be pretty accurately estimated and allowed for, the results of the two determinations often agree very closely. Murlin and Lusk report a series of twenty-two different experiments upon a dog in which the average

difference between the results of indirect calorimetry and the direct measurements was only 0.6 per cent.

The Energy Liberated by Basal Metabolism.—The term, basal metabolism, is used to refer to the minimal rate of energy transformation which can prevail in a healthy, normal animal body. It is the oxidation that still goes on when the "fires" of the body are "banked." It can be measured at a time when the subject is not digesting any food (in man, 14 to 16 hours after the last meal) and when lying perfectly still so that voluntary muscles, other than those of respiration, are entirely at rest and have been resting for not less than half an hour. Under these circumstances, the body is doing no external work and is metabolizing merely to the extent required for maintaining circulation, constant body temperature and the general equilibria of vital processes.

Basal metabolism has been studied in relation to species, age, sex, body weight, conditions of health and other factors. It cannot be determined with great accuracy in all animals because of uncontrollable variations in their muscular activity. In some cases this difficulty has been met by curarizing the animals and measuring their metabolism in a calorimeter which is maintained at constant temperature. Dogs can be trained to lie perfectly still during an experiment. In any case, however, measurements under comparable conditions of minimal activity can be made. The comparison of such measurements, computed as the calories produced per hour per kilo of body weight, shows an apparent wide variation in the basal metabolism of different species of animals. This is true even when the results compared are restricted to those obtained with warm-blooded animals. The smaller the animal, the higher is its basal metabolism per kilo of body weight.

Rubner's Law of Metabolism.—The weight of an animal is not a true index of the amount of actual protoplasm which it contains. In large animals, the proportion of bone, fat, connective tissue, etc., that is, of intercellular non-living material is larger than it is in small animals. Rubner has suggested the hypothesis that the basal metabolism of an animal is proportional to the active protoplasmic mass. This hypothesis has not been adequately tested, although there is some evidence in its favor. Another general law of the rate of basal metabolism was also formulated by Rubner and has been more adequately tested. This law states that the basal metabolism of warm-blooded animals is proportional to the area of the external surface of the body.

In any two bodies of similar shape but different size, the surface of the smaller one is larger in proportion to its size than is that of the larger body. Of two spheres, one twice the diameter of the other, the smaller one has only $\frac{1}{8}$ the volume, but has $\frac{1}{4}$ as much surface as the other. In bodies composed of material of the same specific gravity, their weights are proportional to their volumes; so that the weight of the smaller of

two such similar bodies is less in proportion to that of the larger one than is its surface. Thus, of two animals of the same shape but one having $\frac{1}{8}$ of the weight of the other, the smaller one has $\frac{1}{4}$ of the surface of the other. For example, suppose two dogs to be of the same age and general shape but of breeds that so differ in size that one dog weighs 1 kilo and the other 8 kilos. Their surfaces will be found to be in the proportion of 1 to 4 though their weights are as 1 to 8. The body surfaces of the two are proportional to the cube roots of the squares of their weights ($\sqrt[3]{1^2} : \sqrt[3]{8^2} = 1 : 4$). In any given species of animal, the actual surface can be computed from the weight, provided that a constant factor, peculiar to the species and relating weight to surface, be known. This constant allows for peculiarities in animal shape, such as long limbs or large ears. If S is the surface, K is the constant and W is the weight, then

$$S = K \sqrt[3]{W^2}$$

The determination of the constant can be made only by actual comparison between the weight and the measured surface of an animal. For human beings, certain empirical formulas have been worked out to relate body surface to the weight and height. By the use of one of these formulas, the basal metabolism can be computed per square meter of body surface. This gives a standard of normal basal metabolism to serve for comparison with the metabolic rate under varying conditions of age, diet, health, etc. The following table, from data calculated by E. Voit, shows how uniform is the metabolism *per unit of body surface* in animals of different size, although the metabolism *per unit of weight* varies widely.

CALORIES PRODUCED PER DAY

	Body weight in kilos	Per kilo of body weight	Per sq. m. of body surface
Horse.....	441.0	11.3	948
Pig.....	128.0	19.1	1,078
Man.....	64.3	32.1	1,042
Dog.....	15.2	51.5	1,039
Rabbit.....	2.3	75.1	776
Goose.....	3.5	66.7	969
Fowl.....	2.0	71.0	943
Mouse.....	0.018	212.0	1,188
Rabbit (without ears).....	2.3	75.1	917

Not all of these figures represent strictly basal metabolism but all were obtained with resting animals and are comparable. They serve to show that warm-blooded animals produce about 1,000 calories per day per square meter of body surface.

In cattle, the total nitrogen of the body has been found to be proportional to the surface area. If the total nitrogen content can be considered as an index of the amount of active protoplasm, this observation upon cattle suggests that Rubner's first hypothesis is correct, that is, that the metabolism of the animal is proportional to the total amount of protoplasm in the body.

The Effects of Age, Sex and Health.—Age influences basal metabolism. This factor has been studied in human beings. Except in the case of infants less than one year old, advancing age is accompanied by a general

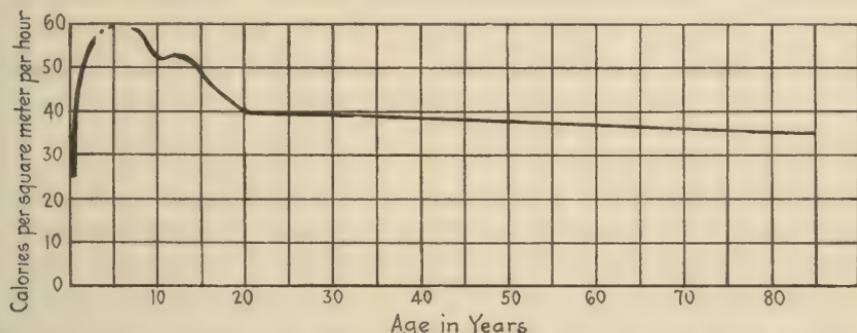


FIG. 170.—Curve showing basal metabolism per square meter of body surface in relation to age of human males. (*After Du Bois*).

tendency to a decrease in the average rate of basal metabolism computed per square meter of body surface. The curve given in Fig. 170 shows the changes in the average basal metabolism with the age of human males. A higher rate occurs during the period of growth. Old age is accompanied by a slowing down of metabolism, a dampening of the fires of life.

The basal metabolism of men is, on the average, higher than that of women. Even pregnancy does not appear to increase the basal metabolism when the metabolism of the fetus is allowed for on the basis of determinations made upon new born children. Lactation is also without significant effect upon basal metabolism.

Many disturbances in health affect basal metabolism. Of these, fevers are the most pronounced. In typhoid fever, for example, the basal metabolism may increase to 40 or 50 per cent above the normal rate. Some hyperthermias (abnormally high body temperature) are the result of a failure in the mechanism for heat loss. These do not necessarily involve heightened metabolism. But fever in the ordinarily accepted sense, especially all infective fevers, are accompanied by increased metabolism. Whether the heightened body temperature is itself responsible, in part at least, for the increase in metabolism or is merely the result of it, is hard to decide. The two phenomena are concurrent. In addition to fever, several kinds of disturbances in the

activity of internally secreting glands cause a rise in basal metabolism. Other disturbances of such glandular functions produce abnormally low rates of basal metabolism. The internal secretions and their effects will be discussed in the following chapter.

The Energy Liberated as a Result of Food Ingestion.—During the 12 to 18 hours after a meal, human heat production is increased above the level of basal metabolism. This is shown by either direct or indirect calorimetry. In herbivores, digestion processes are so prolonged that absorption of food is normally continuous. In them, the basal rate of metabolism is not reached until the animal has been without food during some 40 to 50 hours. Carnivores resemble man in that the effect of eating upon metabolism does not last more than 18 hours and may disappear in 12 hours. This increased metabolism is due chiefly to the specific dynamic action of food. Earlier views attributed the metabolic increase to the work of the digestive system. The secretion of digestive juices and muscular contractions of the stomach and intestines require energy and might naturally be expected to cause an increase of oxidative metabolism with an accompanying increase in heat elimination. Doubtless there is some increase due to digestive activity but its amount is small compared with the increases actually observed after eating. After a protein-rich meal, metabolism may increase above the basal rate to an extent which, if produced by muscular contractions, could occur only during the hardest exercise. The non-striated muscles of the digestive system constitute a very small proportion of the body musculature and act economically, as explained in connection with the physiology of tonus. The digestive muscles are not entirely quiescent even when digestion is not going on, but tend to maintain some rhythmic activity in addition to tonus. Their part in the postprandial rise in metabolism must be very small. Indeed, it cannot be detected when indigestible substances, such as agar, or foods free from protein and carbohydrate are fed to an animal which has been reduced to the basal level of metabolism. Even the ingestion of meat extracts, which stimulate activity of the digestive glands in addition to that of the digestive muscles, does not appreciably raise the metabolic rate. This proves that digestive processes cannot account for an increase in metabolism after eating and shows that the specific dynamic action of food is exerted upon the cells of the body, irrespective of their contractile or secretory activities. This appears to be a case of mass action, in that the mere increased concentration of free amino-acids and carbohydrates, present in the tissues but not synthesized into protoplasm, and the presence of excessive amounts of fats in the blood cause an increase in the rate of oxidative reactions.

As explained above, the introduction of amino-acids, that is, the eating of protein food, exerts the most marked effect. In a dog, for example, metabolism rises from the basal rate to one which is 40 to 90

per cent higher after the eating of 550 gm. ($2\frac{1}{2}$ lbs.) of meat. The higher the temperature of the room in which the experiment is done, the greater is the increase in metabolism as a result of protein feeding. This gives an experimental basis for the popular idea that a high meat diet (protein-rich) is a disadvantage in hot weather. Small amounts of protein show little effect on the metabolic rate and at low temperatures may cause no increase because the body is already metabolizing rapidly.

The effect of carbohydrate, though less than that of protein, is readily measured. When carbohydrate is ingested in amounts above that which the body can convert into glycogen, metabolism is raised. For example, in a man of average weight, the ingestion of 200 grams of glucose may raise metabolism 16 to 20 per cent above the basal level. Larger increases have been observed. The respiratory quotient also rises and may become equal to unity, thus indicating that carbohydrate is being oxidized. Excessive carbohydrate ingestion does not cause an unlimited increase in the rate of metabolism. It can be increased in this way only to a certain rate which is not exceeded because the available carbohydrate, in excess of that used by the stimulated oxidation, is converted into fat. This limiting of the specific dynamic action of carbohydrate is an automatic self-regulating process, characteristic of protoplasm.

The eating of fat, in amounts sufficient to increase the fat content of the blood, causes a rise in metabolism above the basal level. In man, for example, 200 g. of butter increase metabolism 9 to 14 per cent above the basal rate. The maximal increase occurs 6 or 7 hours after eating the fat. This is known to be the time when the maximal rise in the fat content of the blood occurs. With very high fat ingestion, increases of metabolism, reaching levels 20 to 30 per cent above basal, have been recorded. External temperature influences the effect of fat upon metabolism in the same way that it does that of protein. Excessively fatty foods, like protein-rich ones, cause the liberation of heat in the body to a greater extent in hot weather than at lower temperatures. A fall in the respiratory quotient occurs after ingestion of fat by a fasting man or animal so that the actual oxidation of the fat is detected. The extent and duration of the specific dynamic action of fat is limited by the rapid withdrawal of fat from the blood for storage in adipose tissues and elsewhere. It has been shown that fat, sufficient to double the percentage fat content of the blood, can be injected into an animal and yet disappear so rapidly from the circulation that the fat content of the blood decreases, 5 minutes after the injection, to its previous level.

With a normal mixed diet, the specific dynamic effect of all three of the classes of foods, proteins, carbohydrates and fats is exerted. Lusk has shown that their effects are additive. This is illustrated by the following results of one of his experiments upon a dog. Allowing for the fact that the glucose, when given alone, was in larger amount than when given with

Substance fed	Increase of metabolism above basal	
	Calories	Per cent
Glycocol, 20 gm.....	4.9	25
Glucose, 70 gm.....	6.9	30
Fat, 75 gm.....	3.9	17
Sum of separate effects.....	15.7	72
Glycocol, 20 gm. + glucose, 50 gm. given 4 hrs. after fat, 75 gm.....	14.6	64

the other nutrients, the combined effect is seen to be about equal to the sum of the separate effects.

The Energy Liberated During Muscular Work.—Metabolism is increased during muscular work. Part of the energy liberated by the increased oxidation appears as heat. The heating effect of exercise is too obvious to require experimental proof. Any marked rise in the body temperature is prevented by increased loss of heat. This is attained chiefly by the evaporation of sweat but partly by increased radiation. Some of the energy liberated by the increased metabolism during muscular activity is utilized in work. The following table shows the results of a series of experiments conducted by Rubner upon a man of 61 kilos weight. During each of the working periods in this experiment,

Diet and condition	Energy liberated			Remarks
	Total during 24 hours	Increase	Increase due to work	
No food, rest.....	Calories 1,976	Percent	Calories	Practically basal metabolism.
Sucrose, rest.....	2,023	+ 2.4	...	Specific dynamic action of carbohydrate.
Same, with work.....	2,868	+45.2	845	234 calories used for work.
Meat, rest.....	2,515	+27.2	...	Specific dynamic action of protein.
Same, with work.....	3,370	+70.5	855	234 calories used for work.

work was done to the extent of 100,000 kilogram-meters. Its amount was exactly measured by means of an ergometer designed for use with

the arms. The 100,000 kilogram-meters are equivalent to 234 calories, taking the mechanical equivalent of heat as 426.5 kilogram-meters per calorie. The average increase of the body metabolism produced by this amount of work was 850 calories. Of these, 234 were used for work. The mechanical efficiency of the arm muscles, that is, the proportion of the available energy which they utilized for mechanical work, was $\frac{234}{850}$ or 27.5 per cent. Higher efficiency, 33 per cent or more, has been observed in measurements involving work of the leg muscles (p. 314). The remaining 616 calories, produced by muscular work in the above experiment, were eliminated as heat. The experiment shows that the energy liberated as a result of the specific dynamic action of food is not utilized for work, but that muscular contraction causes the liberation of energy in addition to that set free by the action of foods.

As explained in the preceding chapter, protein is not used for muscular work, excepting in so far as it contributes to the available supply of carbohydrate. At least, protein catabolism is not increased by work, so long as carbohydrate or fat is available. The relative amounts of the carbohydrate and the fat used are determined, apparently, by the amount of carbohydrate available. So long as it is plentiful, it is chiefly used. This is shown by the fact that the respiratory quotient is high during muscular work by a normally nourished individual and is raised above the value that it has during a previous resting period. But during prolonged work, the respiratory quotient decreases. This is due to the depletion of the limited carbohydrate supply of the body. Fat is then used in larger proportion than at first. The respiratory quotient may become low enough to show that protein and fat are being oxidized with little or no carbohydrate. For example, soldiers, at the end of a day's march, may show a respiratory quotient of 0.78, although in the morning it was 0.88 or 0.90. Muscles apparently work equally well and with about the same efficiency whether fat or carbohydrate is used; but observations indicate that prolonged work is done at the best advantage when some carbohydrate food is available. This is probably due to its antiketogenic action. The restorative power of sugar during fatiguing muscular work is said to be greater, weight for weight, than that of any other food.

Nervous excitement and mental activity increase metabolism but no measurements of this increase have effectively eliminated the muscular contractions which accompany nervous activity. It is certain that most of the increase in metabolism, which occurs during mental work, is caused by contractions of skeletal muscles. They are active in maintaining a somewhat rigid posture of the body and in other ways, under these conditions. When complete muscular relaxation is maintained, all attempts to detect a rise in metabolism during mental work fail to yield decisive results. The brain constitutes only a small portion of the body weight so that a small rise in its rate of oxidative metabolism would

have little effect upon the sum total of that in the entire body. Such an effect appears to fall within the limits of experimental error. Emotional states cause a marked rise in body metabolism, but this also is attributable to the concurrent muscular contractions.

The Energy Balance.—Comparison between the results of direct and indirect calorimetry permit of the striking of a balance between the energy which is liberated in the body and that which is changed into detectable forms. Indirect calorimetry gives the oxygen utilization and the respiratory quotient. From these figures and the protein metabolism, as shown by urinary nitrogen, the amounts of protein, carbohydrate and fat that are metabolized may be computed. This gives the data for calculation of the total amount of energy liberated during the experimental period. If the heat elimination is simultaneously measured and the muscular work, which is done meanwhile, is performed upon an ergometer, the data are at hand for calculation of the disposal of energy. A balance can then be struck. The energy liberated, according to such calculations, and that measured as heat and work, are equal within the limits of unavoidable experimental error. In many cases, the two results differ by an amount less than 1 per cent of the total. In a series of experiments, such differences tend to cancel each other because the energy actually measured is sometimes a little more and sometimes a little less than that calculated from the metabolism. This is illustrated by the following figures from two of Rubner's experiments.

Energy calculated from metabolism, calories	Energy measured, calories	Difference, per cent
2,249.8	2,276.9	-0.42
4,780.8	4,769.3	+0.43

Two experiments reported by Atwater and Benedict give additional evidence.

Energy calculated from metabolism, calories	Energy measured		Difference, per cent
	As heat, calories	As work, calories	
5,093	4,593	543	-0.84
5,144	4,555	550	+0.75

Such results furnish adequate proof that the living organism operates in accordance with the law of the conservation of energy. They also indicate the importance of maintaining an adequate calorific supply for the animal body if it is to maintain its life.

The Calorific Requirements of Man.—The actual fuel needs of the human body can be calculated from the results of calorimetry. In the case of a man of 20 to 30 years of age, his basal requirement is, on the average, 39.7 calories per hour per square meter of body surface. If he is of average weight (70 kilos) and height (168 cm.), his surface area is approximately 1.8 sq. m. This figure is derived from an empirical formula based upon actual measurements made by Du Bois. For 24 hours the man's basal metabolism is 1,715 calories ($39.7 \times 1.8 \times 24$). Food ingestion brings up the metabolism. This effect varies with the quality and the amount of food and is more pronounced at certain periods of the day than at others. No very exact allowance can be made for this; but numerous calorimetric measurements, especially those made by Benedict, show that, for the entire 24 hours, food increases the metabolism by not more than 10 per cent of the basal rate. If this generous allowance is made for the dynamic action of food, 172 calories are required for this purpose. Further allowances are needed in accordance with the amount of activity. The more muscular work done by the man, the larger are his fuel needs. Benedict has shown that quiet sitting in a chair increases metabolism by 8 per cent above the basal rate. Supposing the man sits quietly reading during 2 hours of the day, an allowance of 11 calories ($39.7 \times 1.8 \times 0.08 \times 2$) can be made for this need. Movements, such as are involved in desk work while seated, cause an increase of 29 per cent above basal rates. Supposing the man to lead a sedentary life, in which some 12 hours per day are spent at work while seated, an allowance of 249 calories ($39.7 \times 1.8 \times 0.29 \times 12$) must be made for this activity. For exercise that he may take, only a roughly estimated allowance can be made. The muscular work involved in walking at an average rate of 2.7 miles per hour has been shown to require about 160 calories per hour. Such exercise is representative of that taken during the time that a man of sedentary habits is muscularly active. If this time amounts to two hours per day, an allowance of 320 calories must be made for it. Adding up these various allowances:

	Calories
For basal metabolism.....	1,715
For metabolism, stimulated by food.....	172
For metabolism, due to 2 hours' reading.....	11
For metabolism, due to 12 hours' sedentary activity.....	249
For metabolism, due to 2 hours' mild exercise.....	320
Total.....	<hr/> 2,467

Computations, such as these, have led to the commonly accepted standard allowance of 2,500 calories per day for the average fuel requirements of a sedentary man. Of course, this requirement varies with different individuals and for the same person on days of differing activity. It also varies with sex, being less for women in general than for men. It varies with age, being less for children and for old people than for younger adults. Calorimetric measurements, continued during periods of a day or more, confirm the calculated value, 2,500 calories, as the average need. It must be very near to the average requirement of the individuals of an urban population. This is shown by statistical data. If the total calorific value of the food dispensed daily in a given city is divided by the total population, the result is very near 2,500 calories per person per day. Even when the varying needs of different individuals are taken into account, all the members of a given population do not receive their proper share. Under-nutrition is recognizable in the slums of most large cities. Over-feeding is also common in many quarters. Food, consumed in excess of an individual's needs, causes fat storage.

The requirements of a laboring man are very different from those of a sedentary person. Statistical evidence shows that farmers consume, on the average, 3,550 calories per day. This is probably not in excess of their actual needs. Such data have been gathered from widely separated countries and do not vary greatly in different localities. Maine lumbermen have been found to use as much as 7,000 calories per day and a man, riding in a six-day bicycle race, showed a metabolism at the rate of 10,000 calories per day. Exposure to cold tends to increase heat production by shivering and especially by stimulating the individual to take more muscular exercise.

The metabolism involved in various kinds of work and athletic exercise has been determined calorimetrically. Typewriting, typesetting, factory machine operation, weight lifting, running, hill-climbing, swimming and some other activities have been investigated. From the results of such determinations, the actual calorific needs of an individual can be calculated fairly accurately. By these observations, it has been shown that training in any given activity not only improves the ability to do it well and with minimum fatigue, but also improves the working efficiency of the muscles. The untrained worker uses more muscles than he needs to and thus wastes more energy than the trained person does.

The requirement during growth is higher in proportion to the body weight than during adult life. This is due to the fact that energy is stored away in the form of new tissue. Rubner has shown that a definite quantity of energy is required to afford a given amount of growth. Growth, sufficient to add one kilo to the weight of the animal requires the same number of calories of food in all species investigated except the human. A child requires about six times as much energy for a given amount of

growth as that required by other animals for the same amount. The stunting effect of insufficient food has been recognized in children as well as in domestic and laboratory animals. Such retarded growth can be made up by subsequent adequate feeding.

The Value of Dietetics.—The practical application of the science of nutrition is found in dietetics. Its practical value has been questioned on the ground that appetite naturally provides for adequate nutrition. But appetite is not a safe guide. This is especially true in the case of children. Appetite is also an unsafe guide for many adults on account of human idiosyncrasies of taste and the tendency to form dietary habits which are sometimes bad. The science of nutrition has important applications in the correct feeding of domestic animals for they are not usually free to choose their own food. Similarly, the feeding of human beings in many institutions, in armies and navies and in any situation, where provisioning on a large scale determines the diet, irrespective of individual tastes, requires a knowledge of the true nutritional needs of man. Choice of food is also limited by poverty so that the poor would be especially benefited by a knowledge of nutrition. Unfortunately, they are usually the people who know the least about it.

The Balanced Diet.—Certain main principles should guide in the selection of an adequate diet. The calorific needs must be fully met and preferably a little exceeded as a margin of safety. The protein requirement must be met. A "balanced diet," containing carbohydrate and fat as well as protein, is important. The value of the carbohydrate has been explained above. Its place can be taken, as it is in the case of carnivores, by a very high consumption of protein food. But such a diet is very expensive because meats, eggs and all typically protein-rich, carbohydrate-poor foods are costly. Moreover, the human body does not appear to be adapted, except when living in an Arctic climate, to the use of such a diet. So long as fat is included in the diet, some carbohydrate is an advantage as tending to insure the proper oxidation of the fat. The special usefulness of fat lies in its high calorific value. The eating of a reasonable quantity of it along with other foods furnishes sufficient energy without making the diet as bulky as it is when proteins and carbohydrates are the chief foods.

The following table, compiled by Lusk, shows three types of diets. That of the Eskimo predominates in protein and fat. Its small amount of carbohydrate is glycogen. It is a carnivorous diet and is suited to a cold climate. That of the Bengali is a predominately carbohydrate diet. It is comparatively inexpensive but is not unquestionably adequate. The average European laborer's diet is suited to temperate climates and is representative of diets that are adequate.

The planning of dietaries to meet any given requirements of total protein supply, total calorie value and distribution of calories is easily

done by the use of tabulated food values such as have been published by the U. S. Department of Agriculture. These values are based upon analyses done chiefly by Atwater and his co-workers.

	Body weight, kilos	Average daily protein intake, grams	Total daily intake, calories	Distribution of calories		
				Protein, per cent	Fat, per cent	Carbo- hydrate per cent
An Eskimo.....	65	282	2,604	44	48	8
A Bengali.....	50	52	2,390	9	10	81
A European (laboring man).....	70	118	3,055	16	17	67

The Optimum Amount of Protein Food.—As explained in the preceding chapter, the actual need of protein to replace "wear and tear" is small compared to the European average standard of 118 g. per day for a man of average size. As an excess of protein above the replacement needs is not used in the adult to form body protein, the question arises as to whether large amounts of protein are an advantage.

Several arguments are urged against a high protein diet. It is supposed to put unnecessary work upon the liver and the kidneys in disposing of an excess of amino-acids and their resulting waste products. There is no adequate proof, however, that the liver and kidneys are inadequate to this task under the conditions of normal health. A high protein diet is apt to contain much meat which yields purine substances and therefore leads to the production of uric acid. The human body is not well equipped to cope with an excess of uric acid, so that gout is more liable to occur as a result of a meat-rich diet than during the use of one which includes little meat and thus tends to be low in protein. Greater resistance to fatigue, both physical and mental, has been claimed by users of low protein diets. In some cases, these claims have been based upon something more than the mere subjective evidence of introspection, being substantiated by improvement in the results of gymnastic tests of strengths and resistance to fatigue during the use of a low protein diet. A lessening of the tendency to protein putrefaction in the intestines and the consequent auto intoxication furnishes what is perhaps the strongest physiological argument in favor of limiting protein consumption to little more than the minimum requirement. A purely economic circumstance is probably the one which most frequently operates to cause people, either voluntarily or involuntarily, to utilize a low protein diet. Under most conditions of complex civilization, protein-rich foods are sufficiently costly to cause marked limitation of their use in a considerable proportion of the population.

Arguments have been urged in favor of a high protein diet. Statistical data indicate that certain progressive and economically successful nations utilize a diet richer in protein than that of certain less successful groups. This is hardly a conclusive argument, since economic success is as apt to be the cause as it is to be the result of the consumption of a high protein diet. But the low protein diet of the people of Bengal, in southern India, a diet composed chiefly of rice with a little fish, does not appear to enable them to do hard work. The Bengalis are smaller, on the average, than are the natives of other parts of India where proteins are eaten in larger amounts and in greater variety. This does not prove, of course, that the amount of protein in the diet has a direct effect upon growth, but it may be one important factor. Adequately controlled experiments on animals do indicate that rapid and satisfactory growth is favored by a high protein diet. The carnivorous diet of the Eskimo does not cause gout or any deficiency in working capacity so that some of the arguments in favor of limiting protein foods do not seem to apply in the case of this high protein eating race. But the stimulating effect of a cold climate must be taken into account. Protein has a peculiar value under such conditions because of its tendency to increase heat production by means of its specific dynamic action. The chief argument in favor of the use of protein in excess of the minimum requirement is that it constitutes a factor of safety, insuring rapid repair of tissue breakdown and having a general effect upon metabolism.

Between these conflicting arguments, an entirely satisfactory conclusion cannot be drawn as yet. One aspect of the question has received especial attention in recent years. This is the value of the quality of the protein as a factor in determining the quantity required. This matter was discussed in part in connection with nitrogen equilibrium. Proteins of animal origin, those of meat, milk and eggs, can make good the tissue "wear and tear" when used in smaller amounts, on the average, than those required for the same results with the proteins of vegetable origin. McCollum and others have shown, however, that supplementary relationships exist between vegetable proteins of different origin. For example, the proteins of the leaf parts of plants make good the nutritive deficiencies of those of the seed parts and a combination of root parts (potatoes, carrots, etc.) with seed parts (including nuts and grains) and leaf parts has been clearly proved to constitute an advantageous diet for even those animals which are not normally herbivorous. Similarly, human individuals have repeatedly proved to their own satisfaction that such a varied vegetarian diet is adequate and, in many cases, they have found it satisfactory when the protein allowance was far below the old standard of 118 g. per day. Usually, so-called vegetarians supplement the diet with milk, a nearly complete food in itself, and with butter and cheese. But even when thus supplemented, this diet is usually a rather

low protein one. When due consideration is given to the quality of the proteins of the food or, to put it in another way, when suitable combinations of different proteins are eaten, the amount required appears to be no more than 50 g. per day for the average human adult. This amount of protein or more is almost sure to be included in any diet which meets the fuel requirement and has sufficient variety to appeal to the appetite. The following day's dietary, representative of those used in Chittendon's low protein experiments, will serve to suggest the quantities of food which furnish an adequate but low protein diet for a sedentary person.

Articles of food	Weight, grams	Fuel value, calories	Protein, grams
Breakfast:			
One shredded wheat biscuit.....	30	106	3.15
One teacup of cream.....	120	206	3.12
One German water roll.....	57	165	5.07
Two 1-inch cubes of butter.....	38	284	0.38
Three-fourth cup of coffee.....	100	...	0.26
One-fourth teacup of cream.....	30	51	0.78
One lump of sugar.....	10	38	
Lunch:			
One teacup homemade chicken soup.....	144	60	5.25
One Parker-house roll.....	38	110	3.38
Two 1-inch cubes of butter.....	38	284	0.38
One slice lean bacon.....	10	65	2.14
One small baked potato (about 2 ozs.).....	60	55	1.53
One rice croquette.....	90	150	3.42
Two ounces of maple syrup.....	60	166	
One cup of tea with one slice lemon.....			
One lump sugar.....	10	38	
Dinner:			
One teacup cream-of-corn soup.....	130	72	3.25
One Parker-house roll.....	38	110	3.38
One-inch cube of butter.....	19	142	0.19
One small lamb chop, broiled lean meat ..	30	92	8.51
One teacup mashed potato.....	167	175	3.34
Apple-celery-lettuce salad with mayonnaise dressing.....	50	75	0.62
One Boston cracker, split.....	12	47	1.32
One-half teacup of bread pudding.....	85	150	5.25
One half-inch cube American cheese.....	12	50	3.35
One demi-tasse coffee			
One lump sugar.....	10	38	
Total.....	...	2,729	58.07

In view of the uncertainty as to what effect a low protein diet might have if continued generation after generation, most physiologists favor the use of an amount of protein in excess of that which appears to be adequate during the life of a single individual. They argue that as the human race, in all parts of the world, forms the habit of consuming, when food supplies permit, more than 100 g. of protein per person daily, this probably indicates a wise provision for a generous margin of safety. It is possible, however, to maintain a fair margin in excess of the minimal requirement and still have a diet which is more economical than the older standard ones. This is done by furnishing about 75 grams of protein per day for an average adult. But any attempt at economy should be accompanied by care in securing a wide variety of protein foods. Those of differing vegetable origin and, preferable, some of animal origin should be included in the diet.

The Quantities of Inorganic Foods Required.—The *requirement for iron* was discussed above (p. 123) in its qualitative aspects. The actual amount of iron needed is not large; but if the supply is deficient over a long period, malnutrition is inevitable. The amount required can be measured by determination of the iron excretion of the body and of the amount of food iron needed to maintain iron equilibrium and normal hemoglobin content of the blood. Numerous investigations, especially those of Sherman and his co-workers, have shown that 15 milligrams of iron per day constitute about the minimum safe supply for an adult of average size. This amount is surely supplied by a diet containing red meats, eggs and green vegetables. Other important sources of iron are nuts, certain fruits and those cereals, such as whole wheat and oatmeal, which are not refined to an extent that robs them of the iron contained in the hulls of the grain. During growth, the iron requirement is higher in proportion to the body weight than it is during adult life. In women, menstruation and pregnancy produce an exceptional demand for iron so that a margin of safety should be maintained. The deficiency of iron in milk has led dietitians to adopt the practice of including green vegetables in the diet of children after the first year of life (p. 124). Older standards depended upon red meats and eggs for the iron supply but newer views suggest that the high protein diet required to furnish adequate iron from these sources is unnecessary and that small amounts of meat and eggs, supplemented by green vegetables, furnish a more economical diet which is probably better nutritionally and insures an adequate iron supply during growth on a predominately milk diet.

The requirement for phosphorus must also be met. This is particularly important for the growing animal if rickets is to be avoided. Herbivores obtain phosphates from the green parts of plants and from grains. Carnivores gnaw bones in sufficient quantity to obtain phosphorus. The young mammal obtains a plentiful supply of phosphorus from milk. The

amount required has been estimated by several investigators from a study of the phosphorus balance of men and animals. In such studies, it is important to examine both the feces and the urine because the excretion of phosphates occurs through the walls of the intestine as well as through the kidneys. This is particularly noticeable in herbivores, in which the major part of the phosphates, whether formed in the body by catabolism of phosphorus-containing compounds, ingested with food or injected into the circulation, is excreted through the intestinal wall and eliminated with the feces. In any case, phosphorus is excreted chiefly in the form of inorganic phosphates with only a small portion, less than 2 per cent of the total, as organically combined phosphorus. According to Sherman, the amount excreted by a man of average size can be balanced by the intake only when the food includes not less than 0.8 to 0.9 gram of phosphorus (equivalent to about 2 grams of P_2O_5) per day. In the case of most individuals, an amount somewhat larger than this is required. In most cases, at least 1.2 grams of phosphorus or 2.75 grams of P_2O_5 are required to maintain phosphorus equilibrium and to furnish a slight margin of safety. This amount or more is liable to be contained in the ordinary varied diet of adults and in the milk-rich diet of children. Meat, fish, milk, cheese, the yolk of eggs, the husks of grains, beans, peas and nuts are important sources of phosphorus; but all foods, with the exception of highly refined sugars, starches, etc., furnish some phosphorus-containing substances.

Much investigation has been undertaken to determine the relative nutritive value of different forms of food phosphorus. The chief ones are inorganic phosphates, phosphoproteins, nucleoproteins, phospholipins and phytin of plants. The products of the digestion of all of these compounds are absorbed and the phosphorus is suitable for utilization by the animal. Of these facts there seems to be no question; but the relative amounts of phosphorus which must be supplied in any of these forms in order to preserve phosphorus equilibrium is not so well known nor is it certain that any one of them can serve as the sole source of food phosphorus. Foods in their natural state contain more than one form. Milk has phosphoprotein (caseinogen), inorganic phosphates, chiefly of calcium, and small amounts of phospholipins, such as lecithin. Meat and fish contain some nucleoprotein and phospholipin but have most of their phosphorus in the form of phosphates, chiefly those of potassium. Egg yolk contains phosphoprotein (vitellin), liberal amounts of phospholipins and small amounts of inorganic phosphates. Grains and other vegetable foods contain some nucleoprotein, phospholipin and inorganic phosphate; but in general and especially in the case of grains, the bulk of their phosphorus is in the form of phytates. These are salts of the phosphorus-rich phytic acid or phytin. Phytin is of unknown constitution but appears to contain six phosphoric acid groups combined with

one carbon-containing group. Phytin seems to be utilized in the animal much as inorganic phosphates are.

Some investigations on man have shown that a smaller amount of phosphorus is sufficient to maintain phosphorus equilibrium when eaten in the form of caseinogen than is required when the phosphorus is ingested chiefly in the form of inorganic phosphates. On the other hand, numerous experiments with rats have indicated that their entire phosphorus needs can be met by inorganic phosphates provided all the other nutritional requirements are adequately supplied.

The requirement for calcium ranks in importance with that for phosphorus. Both of them contribute to the formation of bone as well as to general tissue construction. The value of balanced relations between calcium and phosphorus and between calcium and magnesium was discussed in Chap. V. The actual amount of calcium required can be estimated from the study of the calcium equilibrium of the body. In such studies, as in those of phosphorus equilibrium, the feces must be analyzed as well as the urine. A considerable proportion of the calcium excretion occurs through the walls of the intestine. It appears from the work of Sherman that the smallest amount of calcium which is sufficient for an average adult man is about 0.7 g. (computed as CaO) per day. An amount larger than this minimum should doubtless be furnished. Under ordinary conditions of living, most persons consume more than a gram per day. During growth, and during pregnancy and lactation a generous supply of calcium is particularly important. The sources of calcium are varied. Nearly all foods contain at least some calcium and, with the exception of certain highly refined ones, the amount of calcium is significant. For human beings, however, the most abundant source of calcium is milk. It contains about as much calcium as an equal volume of saturated lime water. Drinking water, when very "hard," contains calcium in amounts sufficient to be of nutritive significance.

The requirement for iodine has not been exactly determined because the amount is so small. According to McClendon, the iodine content of river and other surface waters is comparatively low in parts of the country where the population show a high incidence of those thyroid deficiencies that indicate an inadequate iodine supply. The question will be discussed more fully in the following chapter.

The requirements for other inorganic food substances, such as potassium, sodium and magnesium have not been extensively investigated from the quantitative standpoint. Except in the case of salt hunger, disturbances, due to deficiency or excess of these substances, are not apt to occur.

Preservation of the acid-base balance of the body by suitable adjustment of the diet has attracted much investigation. This work is founded upon the idea that foods, especially proteins, which are metabolized so as to yield potentially acid substances, such as phosphate and sulphate groups,

should be balanced in the diet by foods which are metabolized so as to yield potentially basic substances, such as sodium and potassium ions. But on the whole, these investigations have not disclosed any failure of the body to adjust itself to fluctuations in the relative amounts of acids and bases formed by metabolism. The selective action of the kidneys very rapidly compensates for any tendency toward excess of either acid or base. This action is clearly shown by the corresponding fluctuations in the reaction of the urine. No evidence has been found to indicate that the pH of the blood is altered significantly by changing from a low protein diet to a high protein one, or by any other changes in the acid-base balance of the food. The extreme case of a diet which is practically lacking in all mineral bases does have disastrous or fatal results for laboratory animals, but this extreme condition could not be encountered during the use of any ordinary foods. By far the most important food factor in the preservation of the reaction of the tissues and body fluids is the ketogenic-antiketogenic balance.

The significance of balanced relations between sodium and potassium was discussed in Chap. V.

The Amount of Vitamins Required.—Quantitative determinations of the actual amounts of vitamins required cannot be made because of the lack of any chemical method for their estimation. Comparative results of estimations of the amounts of foods which furnish the several vitamins in quantities adequate for laboratory animals were given in Chap. VIII. From the standpoint of human dietetics, the safe rule is to supply an unquestioned abundance of vitamin-rich foods. Every day experience and nutritional research have clearly demonstrated that any diet containing milk and fresh fruits and vegetables, especially green salads, is bound to be adequate as to its vitamin content. The only types of diets, which are liable to be deficient in one or more of the vitamins, are those composed largely or exclusively of foods prepared from refined flour and other refined cereals, canned goods and various preserved products.

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CHAPTER XXV

CHEMICAL REGULATION BY THE INTERNAL SECRETIONS

Glands, with external secretion, have been described above. They are the glands with ducts such as those which deliver products of secretion to the digestive system. *Ductless glands* also occur. They play a prominent part in animal physiology. The product of such a gland can be delivered only to the blood stream. Such ductless glands are therefore said to produce internal secretions and are called **endocrine glands**. The study of these glands, their secretions and their effects on the body is the science of **Endocrinology**. It is a highly developed branch of physiology with an extensive literature of its own. Text books, monographs and journals, exclusively devoted to this subject, are available. This extensive development has been due to the importance of the rôle which these glands play in the life of the animal. Several of them have been proved to secrete substances of specific and important physiological properties—in short, hormones. Others probably produce hormones even though their specific products have not been actually identified. The regulatory effects of these chemical messengers, governing and correlating activities of the body, render them of prime importance. The ductless glands are even ranked, by some writers, with the nervous system as masters of the animal organism. Some of them are known to be indispensable to life itself.

The important endocrine structures are: The thyroids, the parathyroids, the two functionally and anatomically distinct lobes of the pituitary gland, the pineal gland, the thymus, the adrenal glands, portions of the pancreas and parts of the various sex glands (testis, prostate, ovary, etc.).

The History of Endocrinology.—The marked influence exerted by internal secretions has long been recognized. The effect of castration has been recognized since primitive times. The obvious differences between the capon and the cock, the ox and the bull, the gelding and the horse, the eunuch and the man could not escape notice. That the effects of castration were due to the lack of some chemical substance normally produced in the sex gland was long suspected, but the first formal statement of the idea of internal secretion was written by Johannes Müller in 1833. This idea derived experimental support from demonstrations by Berthold (1849), showing that the transplantation of a cock's testis into a capon transformed the latter into a normal appearing cock. During the decade

following this work, Claude Bernard greatly developed the theory of internal secretion. He showed that sugar was put into the blood from the liver and that this process was one of internal secretion to be distinguished from those of external secretion. Another French investigator, Brown-Sequard (1889) attracted much attention to the idea of internal secretion by his claim to be able to rejuvenate old men by injections of testicular (orchitic) extracts. His, at one time, much advertized results became discredited and were probably in the nature of psychological cures rather than physiological effects. He propounded a working hypothesis which has been very stimulating to physiology. This hypothesis was to the effect that all tissues of the body contribute something to the blood, and that feeding or injection of preparations of animal organs—**organotherapy** or **opotherapy**, should be a widely useful method for treatment of any diseases or abnormalities due to functional derangement. Although this idea is, in general, correct, modern physiology recognizes that the products of some tissues are not useful to the body and are therefore to be regarded as excretions rather than as internal secretions. Marked limitations of the usefulness of organotherapy are also recognized although the great value of certain preparations, such as those made from the thyroid, the adrenals and the pituitary, has been established. It has become convenient to limit the meaning of the term internal secretions to include only the products of the endocrine structures. The modern developments of endocrinology were initiated by the significant work of Bayliss and Starling upon secretin (p. 478). They first suggested the term hormone.

The Thyroids.—A large pair of glands located on either side of the larynx-trachea junction, glands which in some species, especially in man, are fused into what appears to be one gland, constitute the thyroids. The parathyroids are located in the same general region and some of the numerous parathyroid structures are usually imbedded in the thyroid glands. Because of this proximity, much confusion at one time existed as to the respective functions of thyroids and parathyroids. Experimental extirpation of thyroids often involved removal of parathyroids and consequent confusion as to the effects of the operation. It is now clearly recognized that the thyroids and the parathyroids exert distinctly different effects.

The general aspects of the thyroid function are shown by certain diseases which involve it. **Myxoedema** (Gull's disease) is due to an insufficiency of thyroid action. The symptoms include puffy swelling or oedema of parts of the body especially of the hands and face, general depression of all bodily functions accompanied by a lowered utilization of oxygen and usually a depressed mental condition. These symptoms are removed by feeding thyroid glands. (Fig. 171.) Some forms of **goitre** are also due to thyroid deficiency and can be benefited by thyroid feeding.



FIG. 171.—Myxoedema. In the photograph at the right the patient shows complete recovery after 7 months of treatment with thyroid. (From Tigerstedt's "Text Book of Physiology," translated by Murlin, D. Appleton and Co.).



FIG. 172.—Cretinism. In the photograph at the right, the child shows complete recovery after six months of treatment with thyroid. (From Tigerstedt's "Text Book of Physiology," translated by Murlin, D. Appleton and Co.).

The condition known as **cretinism** shows the most striking effects of thyroid deficiency. This condition is congenital and is characterized by poor growth, marked obesity, noticeable softness and flabbiness of all tissues and a tendency to mental defectiveness. Cures, that can only be described as amazing, are obtained by feeding thyroids to cretins. (Fig. 172.) A cretin can be maintained in a normal condition by administration of thyroid at fairly regular intervals throughout life. A condition comparable to cretinism (obesity, depressed oxidative metabolism and general sluggishness) can be produced in experimental animals by extirpation of the thyroids at an early age. A condition comparable to myxoedema can be similarly produced by extirpation of thyroids from an adult animal. Both of these conditions are counteracted by thyroid feeding.

Exophthalmic goitre (Grave's disease) is due to excessive activity of the thyroid. The symptoms include a marked increase in oxidative metabolism as shown by increased rate of oxygen utilization. Figuratively speaking, the patient is burning himself up. Other symptoms are nervous disorders, marked by extreme restlessness and hyperexcitability, a high heart rate, derangement of reproductive functions in women and a protrusion of the eyeballs from their sockets in either sex. It is because of this latter symptom that the adjective exophthalmic is applied to the disease. It is usually fatal if not checked in time. It is greatly alleviated and often cured by operative removal of a part of the thyroid gland or by tying off some of the arteries leading to the thyroid so as to decrease its normally large blood flow.

These various diseases, involving the thyroid, show that it regulates the rate of oxidative metabolism in the body and through this regulation, it indirectly influences the activity of the heart and the nervous system and, indeed, the activities of the whole body. The internal secretion of the thyroid is the kindling which makes the fires of the living animal burn intensely. As all vital processes are dependent upon these fires, that is, oxidations, the thyroid in regulating them, becomes the "pace-setter" for the life of the entire body.

Basal Metabolism as Related to the Thyroid.—The fact that the thyroid influences the general rate of oxidation in the body is demonstrated by measurements of basal metabolism. The simplest of the several measurements used for basal metabolism is the one in which the amount of oxygen utilized in the respiration is determined for a brief but definite period of time. A person who has taken no food during the 15 hours preceding the measurement and who is entirely relaxed during the measurement and the half hour immediately preceding it, should use a certain amount of oxygen per square meter of body surface if he is in perfectly normal condition. This amount has been determined and tabulated for persons of given weight, height and age and is a measure of normal, basal metabo-

lism. Cases of depressed activity of the thyroid show a diminished basal metabolism which may be in some cases more than 30 per cent below normal. Cases of hyperactivity of the thyroid show an increase in basal metabolism from 10 to 40 per cent. Observations on experimental animals from which the thyroid has been removed also show a marked depression of basal metabolism. Both human beings and animals show an increase in basal metabolism after thyroid feeding. These facts show that the general nature of the action of the thyroid is a stimulation of oxidative processes in the body.

Other Effects of the Thyroid.—An increased rate and force of the heart beat, often an irregularity called tachycardia and even hypertrophy of the heart, especially of the left ventricle, result from abnormally high activity of the thyroid or from prolonged overdosage in thyroid feeding. Fatal results, through effects on the heart, may result from excessive thyroid feeding. The formation of new red corpuscles is stimulated by thyroid feeding. Obesity is markedly diminished by thyroid feeding and a general tendency to emaciation is noticed in cases of hyperactivity of the thyroid. Fat is very readily oxidized under the stimulus of the thyroid hormone. Developmental changes such as the metamorphosis of tadpoles and of salamander larvae into adult forms are remarkably affected by the thyroid. This observation was first reported by Gudernatsch and has been abundantly confirmed by numerous investigators. Tadpoles, given small amounts of thyroid with their food, are transformed into tiny frogs no larger than flies. (Fig. 173.) Tadpoles, from which the thyroid is removed by operation, do not metamorphose but grow into tadpoles of abnormally large size. The rate of growth has been supposed to be affected by the thyroid. This claim is disputed by some investigators but in certain species, at least, thyroid feeding does undoubtedly cause a detectable slowing of the rate of growth. Protection of mice against the toxicity of aceto-nitrile was observed by Hunt after feeding of thyroid. This protection does not occur in some other species, for example, rats, and does not indicate a general detoxicating function of the thyroid.

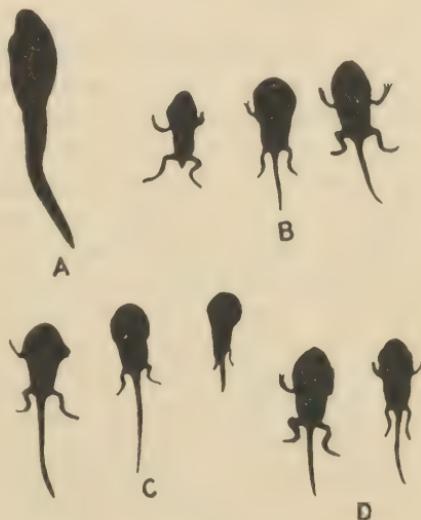
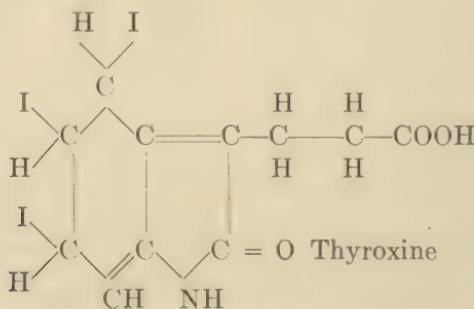


FIG. 173.—Effect of thyroid on metamorphosis of tadpoles, *Bufo vulgaris*. A, control. B, fed on thyroid and muscle. C, fed on thyroid and plants. D, fed on thyroid and pituitary. All animals of the same age. (After Gudernatsch).

The Thyroid Hormone.—The physiologically active substance of the thyroid secretion is an iodine-containing compound. Iodine has long been known to be a thyroid constituent and careful investigations have shown that this element is not present in detectable amounts in any other animal tissues under normal conditions. The amount of iodine in supposedly normal thyroids varies from about 0.1 to about 0.33 per cent of the dried weight of the gland. The amount varies with species, age, season of the year and especially with iodine content of the diet. Some analyses of thyroids from very young animals or children fail to reveal any iodine. Goiterous thyroids are also sometimes lacking in iodine. The thyroid has the power to store iodine and selects from the blood the very minute quantities of this element absorbed with the products of digestion. Many foods, especially sea-foods contain minute but detectable quantities of iodine. Drinking water of most regions contains faint traces of iodine. Drinking water is probably an important source of iodine for a large proportion of the human race. The nature of the compounds which contain the iodine of foods and of water is not known. Whatever these compounds may be, they appear to furnish iodine in forms which the thyroid can store and utilize.

The iodine compounds of the thyroid have been extensively studied. Baumann (1896) prepared a product of the partial hydrolysis of thyroid proteins and named it **iodo-thyrin** or **thyroidin**. It contains 9.3 per cent of iodine. It yields the active hormone of the thyroid, as is shown by beneficial effects of feeding it in cases of deficient thyroid function. Iodine is held in the thyroid in a protein combination which has been separated and is called **thyreo-globulin**. This protein or entire thyroid substance yields the free hormone when hydrolyzed in strong alkaline solution. Kendall (1918) isolated and identified the hormone and proved its chemical structure by artificial synthesis. It is tri-iodo-tri-hydroxyindole propionic acid:



It contains 65 per cent of iodine which is in agreement with its structural formula. It has a structural relationship to the amino-acid, tryptophane. Kendall named this substance **thyroxine**. It is very powerful in producing the physiological effects characteristic of the thyroid action, such as

metamorphosis of larvae, rise of basal metabolism in man and the cure of myxoedema. As little as 10 mgr. of thyroxine has been observed to cause an increase of 30 per cent in the basal metabolism of a woman with myxoedema and, curiously enough, to maintain this effect during several weeks.

Iodine may possibly be converted into a physiologically useful substance in some animal tissues other than thyroid. Swingle has shown that thyroidless tadpoles can be made to metamorphose by giving minute amounts of pure iodine with their food. Potassium iodide is nearly as effective as iodine itself and even iodoform has some effect. Iodates were found to be without effect. These observations suggest that iodine can be transformed into a useful hormone in some as yet underdetermined part of the body. The usefulness of iodides, such as KI or NaI in counteracting the effects of hypothyroid conditions has been established. The dosing of school children twice a year with NaI has greatly checked the prevalence of goitre (hypothyroid type) in cities of Ohio where the frequency of this disease is very high. Iodides are beneficial in cases of cretinism and can be used instead of thyroid feeding.

The Parathyroids.—The parathyroids consist of small yellowish bodies most of which are located near the thyroids. Yet they are distinctly different in histological structure and embryonic development from the thyroids. There are several pairs of parathyroids. One pair is very close to or even embedded in the thyroid. Another pair is found, in most cases, just above (anterior to) the thyroids. Others, the so-called accessory parathyroids, are scattered along the trachea below the thyroids, sometimes down even as far as the thymus or imbedded in it. Because of the anatomical arrangement of parathyroids, experimental or surgical operations upon the thyroids are apt to involve some, at least, of the parathyroids and complete removal of parathyroids is difficult especially if it is to be done without serious injury to thyroids. Technique, adequate to meet these difficulties, has been developed in the course of modern investigations of thyroid-parathyroid physiology. As in the studies of all endocrine functions, two chief types of experimentation are available: (1) Extirpation with observations of resulting effects and (2) administration (feeding and injection) of parathyroid and other preparations to find means of counteracting the effects of extirpation or deficiency of the gland and to simulate its normal effects.

Results of Extirpation of Parathyroids.—After removal of the parathyroids from dogs, they show characteristic symptoms of intoxication. These include great lassitude and a loss of muscular control. About two or three days after extirpation violent tetanic spasms of the muscles set in. Death follows within ten days after the operation. Cats show the same symptoms, but they occasionally recover from the operation. Rabbits vary in their reaction to it. A large proportion of them survive what is

supposedly a complete removal of all parathyroid glands. Rats, on the other hand, are very sensitive to parathyroidectomy and die within a few hours after the operation. Inasmuch as removal of some of the parathyroid glands without complete extirpation, causes temporary symptoms followed by recovery, the inference is that animals which recover from this operation may possess parathyroid tissue of unknown location. In some cases, a partial parathyroidectomy leaves the animal in a condition called "latent tetany." In this condition, any symptoms, such as tetanic spasms, which occurred soon after the operation, have passed away; but the animal is still potentially subject to recurrences of tetanic fits. These are particularly liable to appear in the female during pregnancy. The fatal results or the tetanic fits, which followed some of the early surgical operations for removal of thyroid, are now supposed to have been due to removal of the parathyroids with the thyroids. In modern thyroidectomies, the parathyroids are carefully avoided. Curiously enough, removal of thyroids with parathyroids, that is, a complete removal of both kinds of glands, results in a longer survival of the animal after the operation than is observed after removal of parathyroids alone. Not more than two of the parathyroids can be removed without causing disastrous symptoms in most species of animals and even the loss of two is occasionally fatal. A deficiency of parathyroid function is supposed to account for so-called "infantile tetany" and "gastro-intestinal tetany." These are forms of children's "fits." Partial removal of the parathyroids of young rats, that is, an experimental deficiency is claimed by Leopold and von Reuss to cause a decrease in the total calcium content of the body, although the bones of their operated animals contained in excess of the normal amount. Lack of parathyroids has been found by some investigators to cause a decreased concentration of calcium in the blood and the brain tissues, but these results have not been obtained by all who have tried such experiments. An increased excretion of calcium either with the urine or the feces or both seems to have been observed unfailingly after parathyroidectomy. Corresponding to these disturbances in calcium metabolism, defective bone growth and poor calcification of teeth have been observed after partial parathyroidectomy in young rats. Phosphate metabolism also appears to be disturbed. A distinct decrease in the total phosphate excretion is accompanied by an increased phosphate concentration in the blood, a retention of phosphates. There is also a tendency to a change in the reaction of the blood, an acidosis with diminished CO_2 combining power. As is usual in acidosis, the excretion of NH_3 , in the urine, is markedly increased, both absolutely and in comparison with total nitrogen excretion. As might be expected, acidosis is also accompanied by defective carbohydrate metabolism which may be the immediate cause of the acidosis. The sugar content of the blood falls to about half of its normal value. Utilization of carbohydrate

is poor and the power of glycogen formation appears to be impaired. The disposal of the sugar which disappears from the blood has not been explained. It is not usually found in the urine.

One effect of parathyroidectomy has attracted much attention in recent years. This is the appearance of **guanidine** or **methyl guanidine** or both in the urine and feces and in the blood. This phenomenon, first described by Koch, has been observed by several investigators. These substances are known to be very toxic. They probably do not arise in significant amounts in the normal animal. Only the merest traces of them are found, ordinarily, in urine. But they have been found, in significant amounts, in the excreta of children with infantile tetany.

Means for Counteracting the Effects of Parathyroid Removal.—All the symptoms following parathyroidectomy and especially the violent tetanic spasms or fits can be temporarily checked by injection of parathyroid extracts. Complete cures have never been produced by this means and only slight prolongations of the life of the operated animal. Apparently, a continuous functioning of the parathyroid gland is requisite for the life of animals. Grafts of parathyroid tissue in dogs have been made successfully by several experimenters. The grafted gland protected the animals from the effects of parathyroidectomy.

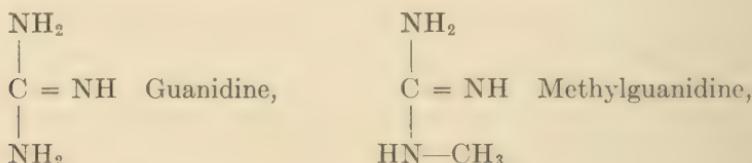
McCallum and others have shown that the injection or feeding of calcium chloride or of other soluble calcium salts causes an immediate cessation of the violent spasms of a parathyroidless animal without, however, prolonging its life. The spasms return when the effect of the calcium wears off. Strontium and magnesium similarly counteract the tetany.

A parathyroid tetanic spasm can also be abolished by partially bleeding the animal and replacing the lost blood with isotonic salt solution or with blood from a normal animal.

The Nature of the Parathyroid Function.—Several theories have been proposed to account for the indispensable rôle of the parathyroids. The spectacular results of administration of calcium, strontium and magnesium salts suggested that the parathyroids produced some hormone that was necessary to preserve the all important balance between calcium and other metallic ions in the body. The tendency to defective calcification in parathyroidless rats agreed with this idea. Abnormally high elimination of calcium in the urine and diminished calcium content of the blood have been observed after parathyroid removal. In spite of these facts, regulation of calcium metabolism by means of the parathyroids cannot be regarded as their sole or chief function. The mere fact that bleeding gives relief from parathyroid tetany shows that something in addition to calcium deficiency is concerned in the effects of parathyroid removal. Moreover, calcium salts have a general sedative or soothing effect on all

animal tissues and might readily abolish tetany through a localized effect on the nervous system and on the muscles.

Another theory of parathyroid function has been suggested by Koch and advanced by Noel Paton. It is based upon the fact that guanidine and methylguanidine appear in the blood and the urine after parathyroid removal and during infantile tetany. This suggests that the parathyroids are concerned in preventing the presence of these very toxic substances in the normal, healthy animal. This idea is further substantiated by the production of typical tetany symptoms as shown by Paton and others after injection of either of these substances. The formulas of guanidine and methylguanidine:



show their relationship to the amino-acid arginine and to creatine and creatinine. These relationships suggest that the appearance or non-appearance of guanidine and methylguanidine in the body will be determined by the character of protein metabolism. The eating of protein foods does, indeed, cause increased violence and frequency of tetanic spasms in parathyroidless dogs and markedly hastens their death. The inference is that an internal secretion derived from the parathyroid glands so regulates protein metabolism that these poisonous waste products are not normally produced in harmful quantities. Some toxic material is certainly present in the blood of parathyroidless animals. Such blood, transfused into the blood of a normal animal, causes tetany.

Watanabe has shown that the effects of injections of salts of guanidine into normal animals include the same derangements of metabolism that occur after parathyroid removal. Even before tetanic spasms are produced, as a result of these injections, the blood sugar is lowered, blood calcium is decreased and calcium excretion increased, phosphate retention occurs, and acidosis with high ammonia excretion is observed. These conditions tend to be especially aggravated when guanidine is administered in amounts sufficient to cause tetanic symptoms or death. Watanabe's observations indicate that all the disturbances, which follow parathyroid removal, are the result of the presence of guanidine and its derivatives in the body and that the primary function of the parathyroids is to prevent the presence of these poisons. Whether they do this by so regulating protein metabolism as to prevent guanidine formation or by detoxicating guanidine after it is actually produced remains to be investigated.

A practical application of knowledge of parathyroid functions is found in the use of parathyroid gland as a cure for infantile tetany. Although this therapy is not sufficiently unfailing as to be called an established cure, many successful results have been reported. The value of a low protein diet in such cases of tetany has been established.

The Thymus.—The thymus is a large gland which surrounds the trachea at its lower end. It is commonly called sweetbread when used as a table delicacy. This gland disappears after puberty. Its chemical composition is unique. It contains a larger proportion of nucleoprotein than does any other animal structure.

The effects of removal of the thymus from young animals are not well established. Reports on such experiments are conflicting and, in some cases, directly contradictory. Marked effects on bone growth were reported by Basch, but his work has not been substantiated. It is possible that some of the fatal results, the failures of growth and the cases of defective bone development, that have been reported after removal of the thymus, could have been avoided if a diet adequate in its vitamin content and its inorganic salts and all other nutritive requirements, were supplied. The physiology of the thymus has not been adequately investigated since the development of modern knowledge about nutrition. In the absence of conclusive evidence the function of the thymus gland cannot be positively stated. The fact that it tends to disappear after sexual maturity suggests that it has something to do with the development incidental to puberty. The persistence of the thymus throughout the period of growth has long been regarded as presumptive evidence that this gland was in some measure a regulator of the growth process. More recent work has shown however that some species of animals, deprived of the thymus even at an early age, can be reared to full and apparently normal growth provided an adequate growth-promoting diet is supplied. In connection with these observations, the fact that the thymus is alone an adequate growth-promoting diet for some species of animals seems significant. Such evidence suggests that the thymus is a storage place for certain food materials that are useful in growth processes and are especially required during the period of the development of sexual maturity. Uhlenhuth has shown that the feeding of thymus to salamander larvae, prior to the time that their parathyroids develop, brings on tetany. This suggests that the thymus materials are stimulating to some phase of protein metabolism. The rich supply of nucleoproteins in thymus would tend to yield large quantities of arginine, for this amino-acid is a large component of thymus nucleoprotein. The development of tetany after thymus feeding in the absence of functional parathyroids may be due, possibly, to the production of guanidine and methylguanidine from arginine. Although the thymus is not necessary for the process of metamorphosis any more than it is for the processes of general growth

and development, nevertheless, thymus feeding hastens the effects of the thyroid in bringing on metamorphosis of amphibian larvae.

The Pituitary.—This gland is known by several different names. Hypophysis, hypophysis cerebri, pituitary body and pituitary gland are names often used interchangeably. Some writers use the term hypophysis for the posterior lobe of this organ and the term pituitary for the rest of it. We shall use the term pituitary for the entire organ.

The pituitary is a small organ upon the base of the brain just back of the optic chiasma. It has three distinct parts: (1) The *anterior lobe*, (2) the *posterior lobe*, (3) the *pars intermedia*. The anterior and posterior lobes are distinctly different in structure and in embryonic history. The anterior is glandular in structure and is an outgrowth of cells on the dorsal side of the buccal cavity. The posterior lobe resembles nervous tissue in structure and is a downgrowth of certain cells in the floor of the third ventricle of the brain. The pars intermedia is partly like the anterior and partly like the posterior lobe. The pituitary occurs in all vertebrates and is represented by an organ of similar structure and supposedly analogous function in many invertebrates.

The pituitary has excited interest and attention since early times. Aristotle wrote about it. Diemerbroick (1686) suggested that it probably secreted something into the third ventricle and this led to the supposition that it even produced the entire cerebro-spinal fluid. Engel (1839) noticed apparent relationships between pituitary disease and abnormal sex development. Brown-Sequard (1869) stated his belief that the pituitary formed an internal secretion. Marie (1889) suggested that an abnormality of the pituitary caused growth to gigantic size and also produced the peculiar overgrowth of certain bones so as to result in the misshapen limbs and the massive jaw which are characteristic of the disease *acromegaly*. Later work has tended to confirm Marie's theory and has further indicated that pituitary deficiency is associated with dwarfism.

The Effects of Extirpation of the Pituitary.—Removal of the pituitary is a very difficult operation. The early results were confusing because of incidental brain injuries. The most successful operations were those done by Cushing and his co-workers and by Aschner. It was at one time believed that complete removal of the pituitary is fatal, but some doubt of this now exists. Fatal results might well be due to injuries to the nervous structures adjacent to the pituitary. Some of Aschner's dogs lived more than a year after an apparently complete extirpation of the pituitary and their death was attributed to disease infection as the immediate cause.

Removal of the pituitary from adult dogs results in no marked disturbance of health. The animals show a general tendency to lay on body fat. Their genital glands show a tendency to fatty degeneration (*dysplasia adiposo genitalis*). Another effect is an apparently lowered resistance to disease. Many of the operated animals die of pneumonia

or other infection. Removal of the pituitary from female dogs during pregnancy causes abortion. The operation causes, in both sexes, certain symptoms of nervous depression, especially depression of the functioning of sympathetic nerves with tendency to lowered blood pressure. Oxidative metabolism is lowered as shown by the respiratory exchange. Protein metabolism is also modified as shown by an abnormally low nitrogen excretion during a period of starvation.

Removal of the pituitary from young dogs causes a remarkable stoppage of growth. (Fig. 174.) The animals become very obese and show a



FIG. 174.—Effect of removal of the pituitary. The two dogs are from the same litter. Both are males. The one at the left was operated on (complete removal of pituitary) at the age of 10 weeks. Picture taken at the age of 1 year. (Aschner).

retardation of the development of the second set of teeth, of the hair growth and of bone development. They show persistent sexual infantilism with failure of functional activity (sperm production) in the testis. The thymus persists long after it would have disappeared from a normal animal. The animals show general defective mental development. They are not as responsive or teachable as normal dogs. The general level of oxidative metabolism is lowered.

All of the above results, both on adult and young dogs, can be obtained if only the anterior lobe of the pituitary is removed. Extirpation of the posterior lobe alone has not been extensively tried. The general conclusions from such experiments are that the posterior lobe has no relationship to growth or development. There is one unique result of removal of the posterior lobe. This is the secretion of abnormally large volumes of

urine. This condition (polyuria) is accompanied by a consuming thirst (polydipsia) and consequent ingestion of large quantities of water to compensate for that removed by the kidneys. The condition is apparently identical with the disease which is called *diabetes insipidus*. Inasmuch as sugar does not appear in the urine in these cases, diabetes insipidus is not to be confused with true diabetes. It has not been shown conclusively that polyuria is a direct result of removal of the posterior lobe rather than an effect of injury to nervous structures adjacent to the pituitary. Another effect is that exerted upon carbohydrate metabolism. Animals, from which the posterior lobe has been removed, can receive larger injections of sugar without having glycosuria than can a normal animal. As commonly expressed, the "carbohydrate tolerance" is increased.

Physiological Effects of Pituitary Extracts.—The injection of extracts of the posterior lobe cause a distinct rise in blood pressure. This was first shown by Howell. Although the rise is not as marked as that produced by the hormone of the adrenal gland, it is more persistent. Such injections also cause a marked stimulation of milk flow in the lactating female. This was first shown by Ott and Scott. It has been demonstrated on cats, goats, rabbits and human beings. This effect, however, is not a real increase in milk production. Although the mammary gland yields a large outflow of milk shortly after the injection, the subsequent activity of the gland shows a compensatory diminution, so that the final result of administration of pituitary extract shows no increase in the total milk production. The inference is that the pituitary hormone excites the contractions of involuntary muscles in the mammary gland so as to squeeze out milk at a rapid rate. This seems all the more probable since the effect of pituitary in causing rise of blood pressure is also due to contraction of involuntary muscles, those in arterial walls. Pituitary extracts cause violent contractions of the muscles of the pregnant uterus and are therefore used for injection to hasten labor. Commercial preparations for this purpose are sold under the names *pituitrin* or *hypophysin*. The pituitary causes contractions of still other involuntary muscles, those of the urinary bladder and of the intestine. The pituitary presumably furnishes something which restrains excretion of water by the kidneys. At any rate, the disease of diabetes insipidus, which is presumably due to defective action of the pituitary, has been successfully treated by administration of pituitary preparations. These cause a marked decrease in the enormous volumes of urine which are characteristically produced in this disease. All of these effects are produced by extracts of the posterior lobe.

Injection or feeding of extracts of the *anterior* lobe cause detectable effects upon growth. Robertson has reported extensive feeding experiments with white mice. Those given pituitary preparation grew for a longer period at a slower rate than control animals and became sturdier,

heavier specimens. The anterior lobe of the pituitary causes more rapid growth of dogs and tends to produce greater sexual development.

Abnormalities of the Human Pituitary.—Tumors and similar growths on or near the pituitary have been observed, not infrequently, in human beings. There are also a few recorded cases of injury to the pituitary, bullet wounds, etc. Such abnormalities might be expected to cause diminished activity of the pituitary—a hypopituitary condition. A number of such cases have been associated with marked obesity, defective or incomplete growth and persistent sexual infantilism. Dwarfs have been known to have especially small pituitary glands. On the other hand, giants are reported to have, in some cases, especially large pituitary glands. Gigantism sometimes appears as a result of a resumption of growth in adults. Accompanying headaches and certain nervous symptoms indicate some irritation which excites the pituitary to hyperactivity. In some cases, these indications have been confirmed by surgical or post mortem findings of tumorous growths near the pituitary. Another curious effect sometimes accompanies gigantism or may occur without it. This is called acromegaly. It consists of a marked enlargement of the bones of the hands and feet. In some cases, especially those reported by Cushing and others following his technique, stoppage of acromegaly and of other symptoms accompanying gigantism has been produced by operative removal of tumors from the pituitary.

Hormones of the Pituitary.—From the posterior lobe, Oliver and Shäfer prepared a crystalline substance, called **pituitrin**. Its composition has not been established. It shows a resemblance to **histamine** which is the product of partial oxidation of the amino-acid, histidine. Pituitrin is like histamine in certain of its physiological effects, such as stimulation of uterine contraction, but is not identical with histamine in all of its effects. From the anterior lobe a substance called **tethelin** has been prepared. It is a phosphorized, nitrogen-containing lipoid, one of the phospholipins. Its discoverer, Robertson, used it in feeding experiments with mice and showed that it had effects upon growth similar to those exerted by the entire lobe. It is probably a true hormone influencing growth. The chemical constitution of tethelin has not been completely established.

Internal Secretion of the Pancreas.—Although the pancreas has long been described as one of the digestive glands producing a typical external secretion, it is, in addition, an internally secreting organ. Certain of its cells, known to histology as the **islands of Langerhans**, are so located that the products of their activity do not get into the pancreatic duct but must be delivered to the blood stream. The internal secretion which they produce is necessary for the proper regulation of sugar metabolism. The failure of this secretion is probably the cause of diabetes. This disease is marked by a disturbance in the normal sugar regulation. The sugar content of the blood is distinctly above the limits of normal fluctuation

and may even be as high as 0.25 per cent of the blood. Corresponding with this, sugar appears in the urine (glycosuria). The power of the body to utilize sugar in the ordinary oxidative processes is greatly impaired, if not entirely lost, in diabetes and the consequent disruption of metabolism causes a tendency to acidosis which becomes very severe in advanced stages of the disease. It is the immediate cause of death from diabetes.

Experimental Diabetes.—The relationship of diabetes to the pancreas has been shown largely by the production of artificial diabetes by removal or other experimental treatment of the pancreas. The famous experiments of Von Mering and Minkowski (1889) first showed that removal of the pancreas from dogs caused hyperglycemia, glycosuria and all other typical symptoms of diabetes. The result of removal of the pancreas (pancreatectomy) is always fatal. Hedon later showed that a piece of pancreas grafted into the abdominal wall, at the time of or before pancreatectomy, prevented the appearance of any diabetic symptoms. The entire pancreas is not needed to prevent diabetes. This is shown by the fact that when three-fourths or in some cases even four-fifths of the pancreas is removed the remaining small portion is sufficient to prevent diabetes in dogs. It has also been shown that only the islands of Langerhans are necessary for preventing experimental diabetes. This conclusion is based on experiments in which the pancreatic duct is tied off or, better still, blocked with a plug of paraffin. This procedure causes a subsequent degeneration of all of the pancreatic cells that secrete pancreatic juice, that is, the externally secreting part of the pancreas. The islands of Langerhans do not degenerate and diabetes is not produced. Any severe injury to the pancreas, such as painting it with iodine or with acids or cauterizing it, causes experimental diabetes.

Inasmuch as experimental diabetes is so clearly the result of the lack of functional power of the islands of Langerhans, the natural inference is that diabetes, in human beings, is the result of a deficiency of these structures. Confirmatory evidence is not lacking. Numerous autopsies after death from diabetes have shown abnormality of the island tissues. Their cells often show what is called a hyaline degeneration. The significance of these observations has been questioned by some investigators because island degeneration cannot always be detected after death from diabetes. Such negative observations, however, need not be regarded as contradictory to the general theory, so well established by experimental work. In the complex interaction of the organs regulating sugar metabolism, the islands of Langerhans might fail in their function without showing structural degeneration although it must not be forgotten that some structure other than the islands of Langerhans may also produce diabetes by a malfunctioning. In this connection, the famous researches of Claude Bernard (About 1860) are of significance. He

showed that injury to a certain point in the floor of the fourth ventricle of the brain causes diabetes. This point is called the **diabetic center**. The operation for producing diabetes by puncture of this center is called **piqûre**. It evidently causes excitation of nerves which influence blood sugar regulation. It is indeed well established that excitation of the splanchnic nerves that are connected with the liver causes a temporary diabetes. This is due to a rapid breakdown of liver glycogen into glucose.

The Mechanism of Sugar Regulation.—In the normal animal, sugar absorbed from the intestine is largely converted into glycogen in the liver. This process prevents excess of sugar in the blood. The reverse reaction, conversion of glycogen to sugar, maintains the normal level of sugar concentration in the blood when sugar is not being absorbed from the intestine. The first of these reactions, formation of glycogen, is in some way dependent upon the internal secretion of the pancreas. This is indicated by the observations of Kleiner and others that an emulsion of pancreas, injected into diabetic dogs (depancreatized), causes a decrease in the blood sugar. More conclusive proof is furnished by the observation that the perfusion of sugar-containing blood through the liver of a depancreatized animal or through an isolated, surviving liver does not cause glycogen storage in the liver, but such a perfusion *does cause glycogen storage if blood from the pancreas mixes with that which passes through the liver*. Probably a hormone from the pancreas favors the production of glycogen in organs other than the liver, for example, in muscles. The breaking down of glycogen into sugar is controlled, in part at least, by the nervous system, which so regulates this reaction as to maintain a supply of blood sugar adequate for oxidative metabolism of the body in general and of the muscles in particular.

The pancreatic hormone may possibly play another rôle in addition to that of promoting glycogen formation. In the absence of a functional pancreas, sugar is not utilized properly and in advanced diabetes is not utilized at all for oxidative metabolism, even in muscles. That some hormone from the pancreas is necessary for sugar oxidation is indicated by experiments of Starling and his co-workers. They have shown that the heart of a depancreatized dog removes distinctly less sugar from blood perfused through it than does the heart of a normal dog. Furthermore, the addition of pancreas extracts to the blood, used for perfusion, restores the power of the heart muscle to utilize sugar. In view of such observations, the theory has been advanced that the pancreas hormone has two functions: (1) That of favoring glycogen production and (2) that of facilitating sugar oxidation. It is possible that these two apparent functions are really only one. Perhaps sugar cannot be oxidized, at least in muscle metabolism, without being first transformed into glycogen. The above reported work of Embden, indicating that glycogen and a hexose phosphate are links in the chain of oxidative transformations in

muscles, strongly suggests that sugar is changed to glycogen before it is utilized.

Although the pancreatic hormone is indispensable for sugar utilization, it is by no means the only one concerned. The hormone of the thyroid, the parathyroid and the pituitary were mentioned above as substances that affect metabolism in general. They incidentally influence sugar oxidation. Hormones of the adrenals and the sex glands will be shown below to be also concerned in sugar utilization.

Treatment of Diabetes.—The success in treating thyroid deficiency by administration of thyroid preparations would make the cure of diabetes by pancreas preparations seem a possibility. Feeding and injecting of pancreas extracts have been, for the most part, without success. The pancreatic hormone apparently produces only transient effects, so that a continuous supply of it is required. Possibly, grafting of pancreas into a diabetic would be effective, but no successful transplants have been reported for human beings. The probable cause of the ineffectiveness of the injection of pancreas extracts has been suggested by the work of Collip, Banting and McLeod. They have shown that the pancreatic digestive enzymes, especially trypsin, cause a destruction of the sugar regulating hormone in extracts of pancreatic tissue. They found that, after ligating the pancreatic duct of a dog and allowing it to survive the operation long enough to permit complete degeneration of the externally secreting cells of the pancreas, the remaining undegenerated islands of Langerhans yielded a preparation which was unquestionably effective in enabling an experimentally diabetic animal to utilize sugar. The applicability of these results to the cure of human diabetes is being tested. Preparations, made from the pancreas for this purpose, have been named **insulin**. Collip, Banting, McLeod and their co-workers have shown that normal pancreatic tissue can be used as a source of insulin, if treated, immediately after removal from recently killed animals, with mineral acid in concentration sufficient to quickly destroy digestive enzymes. The chemical nature of insulin is under investigation. It has been prepared by the above investigators in partially purified form in which it shows only traces of protein material. When so prepared, very small amounts of it lower the blood sugar concentration of normal animals to a marked degree (0.04 per cent) which is accompanied by serious disturbances, including convulsions, and is apt to be fatal unless counteracted by feeding carbohydrate or injecting glucose. The well-established methods for treatment of diabetes depend on dietetic and general hygienic measures. The main principle involved in these methods is a limitation of carbohydrate consumption so as to avoid the disastrous effects of hyperglycemia until the body makes a spontaneous recovery of the power to use carbohydrates in normal amounts. But, during the dieting, a small amount of carbohydrate food appears to be an advantage as tending

to aid in preserving the ketogenic-antiketogenic balance of the body. Periods of fasting, lasting from 2 to 10 days have been found to be helpful in enabling the body to regain some measure of carbohydrate tolerance.

The Adrenals.—A pair of small glands, which in the human adult have an average weight of from 6 to 7 g. each, constitute the adrenals. As their name indicates, they are located just anterior to the kidneys, sometimes in contact with them. The adrenals are very plentifully supplied with blood and are innervated by a branch of the splanchnic nerve. The gland is composed of two portions, the cortex and the medulla. The cortex is derived, embryologically, from the same group of cells as the kidney. The medulla has an entirely different development. It is derived from the same embryonic structures as is the sympathetic nervous system and is largely composed of nerve-like tissue. The medulla has a peculiar histological staining property. Parts of it become yellow after treatment with formalin and potassium dichromate. It is therefore called **chromophil tissue**. With the exception of a few cells of sympathetic ganglia, other chromophil tissue does not occur. Inasmuch as the active principle, the hormone, obtained in extracts of adrenal glands, is quantitatively proportional to the amount of chromophil substance, the latter is supposed to be the source of the hormone.

The adrenal glands are sometimes called the suprarenal capsules or suprarenals. Their active principle is variously named adrenine, adrenaline, suprarenine or epinephrine.

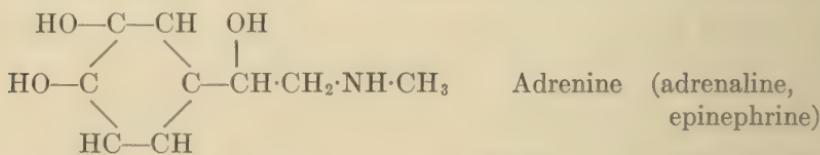
Addison's Disease.—The earliest recognition of the indispensability of the adrenals was given in Addison's description (1855) of an obscure malady, characterized by extreme muscular weakness, susceptibility to fatigue, nervous depression, great digestive irritability and a tendency to pigmentation of the skin in the form of large brown spots. The disease is fatal. Autopsy shows a degenerated condition of the adrenals. The failure of their function was suggested by Addison as the cause of the disease. As first shown by Brown-Sequard (1856) and abundantly confirmed by others, adrenalectomy causes symptoms of complete prostration resembling Addison's disease and has a fatal result in various species of experimental animals, including monkeys, cats and dogs. Adrenalectomy would probably be fatal to man. Tuberculous and other pathological lesions of human adrenals have been found at autopsy after death from Addison's disease. Adrenalectomy is survived by about 20 per cent of rabbits, possibly because they possess sufficient chromophil tissue in their sympathetic ganglia to suffice in the absence of adrenals.

Adrenals and the Autonomic System.—Although the autonomic nervous system commonly connects with effectors only through post-ganglionic fibers, the splanchnic autonomies reach the adrenals as pre-ganglionic fibers. In this way, the adrenals stand in the relationship of sympathetic ganglia to the autonomic system. This is perhaps not

strange considering the embryonic origin of the adrenal medulla. The latter is thus, in one sense, a nervous structure although in another sense, it is glandular. Gaskell has suggested the theory that the chromophil nerve cells of the ganglia of invertebrates represent the common ancestors of adrenaline-secreting cells and of the sympathetic nervous system of the vertebrates.

The close relationship between the adrenals and the nervous system is further shown in the nervous control of adrenal secretion. Electrical stimulation of the splanchnics causes a marked excitation of secretion of adrenaline which can be detected and estimated in the blood of the adrenal vein by means of delicate chemical or physiological tests. Folin, Cannon and Denis developed a colorimetric method for its estimation; Meltzer used the pupillary constriction of the frog eye as a measure of adrenaline; the effects of adrenaline upon various kinds of non-striated muscle and in cardiac muscle have been employed for these estimations. By all these methods the nervous excitation of adrenaline secretion has been abundantly proved. Cannon showed that the reflex excitation of adrenals is also effective. This is particularly true during emotional states. Fear, pain and rage produce marked increases in adrenaline secretion accompanied by all the bodily changes that result from injection of adrenaline. Certain of the physical accompaniments of these emotional states, especially changes in blood pressure, do not occur if the adrenal circulation is tied off prior to arousing the emotional state.

Adrenine.—This hormone has been of especial physiological interest because it was the first one to be isolated in pure form and chemically identified. In the form of a derivative it was first obtained by Abel, but in actual isolated form it was obtained simultaneously, though independently, by Aldrich and by Takamine in 1901. The work of these investigators and others, work which has included artificial synthesis of adrenaline, has proved it to be ortho-pyrocatecol-ethanol-methylamine.



This formula indicates its slight chemical relationship to the amino-acid tyrosine but the relation is not sufficiently close to furnish any clue to the metabolic origin of adrenaline.

The Physiological Action of Adrenine.—The most striking adrenaline effect is the very marked *rise in blood pressure* first demonstrated by Oliver and Shäfer (1894) who used extracts of crushed adrenal glands. It has been abundantly shown that the immediate cause of this rise in blood pressure is a marked constriction of arteries especially of the small

ones, the arterioles. The rise is sharp and sudden and is produced by very minute quantities of adrenine, as little as 0.001 mgr. causing a perceptible effect when injected into a dog or cat. This effect is very transient, probably because adrenine is quickly destroyed by oxidative metabolism. Adrenine disappears from blood exposed to air and can readily be destroyed by oxidizing agents, such as hydrogen peroxide.

Very small amounts of adrenine and, under some conditions, larger amounts cause a *fall in blood pressure*. From the combined results of the work of many investigators, the explanation of this depressor effect appears to be found in vasodilatation in the skeletal muscles. Adrenine produces this effect in an indirect way, namely by an inhibitory action upon the sympathetic ganglia from which emerge the post-ganglionic fibers that control vaso-constriction in skeletal muscles. The resulting vasodilatation tends to lower the general blood pressure; but this is masked, during the action of sufficient amounts of adrenine, by the constriction of the arteries of the visceral organs and the skin. The combined effect, in any case, is a shifting of part of the blood supply of the viscera and skin into the skeletal muscles. This is an important aid to violent muscular exertion. Both dilator and constrictor effects of adrenine are markedly influenced by the condition of the animal: Previous level of blood pressure, depth of anesthesia, presence or absence of functionally active thyroids and parathyroids, the pH of the blood and the presence of certain drugs.

Adrenine has a tendency to cause a *slowing of the heart beat*. This effect is produced reflexly through the cardio-inhibitory center. The rise in blood pressure, acting upon the depressor nerve (p. 289) is an adequate stimulus for this effect upon the heart. Adrenine also exerts *an effect favorable to excitation of the heart muscle*. This is shown by the action of adrenine upon a heart of which all nervous connections have been destroyed. The result is more powerful contractions and increased rate of the heart.

Adrenine not only affects the action of arterial and cardiac muscle, but also *influences the contractions of non-striated muscle in general*. It causes contraction of the dilator muscle of the pupil of the eye; contraction of the non-striated papillary muscles in the skin, thus producing erection of the hairs of the dog's back and the cat's back and tail and "gooseflesh" in the human skin; it causes a cessation, that is, inhibition of gastro-intestinal contractions. In short, adrenine exerts effects upon every sort of non-striated muscle, provided it is enervated by autonomic fibers. A given muscle will be excited by adrenine if autonomic fibers excite it and will be inhibited by adrenine if autonomic fibers cause inhibition. Adrenine really acts upon some substance at the terminations of sympathetic or other autonomic nerves. It does not act upon the nerve trunks themselves. This is shown by the fact that even when complete degeneration has set in after the cutting of sympathetic nerves, adrenine still acts

effectively upon non-striated muscle. The actual seat of the reaction must be upon some substance lying between the visible nerve ending and the muscle substance. This is commonly spoken of as the "receptive substance." Adrenine acts upon this substance so unfailingly that the excitation or inhibition of non-striated muscle by adrenine is used satisfactorily as a method for the detection of endings of autonomic nerve fibers.

Injections of adrenine cause *an increase in the sugar of the blood*. Cannon and his co-workers have particularly developed this phase of adrenine effects. They have shown that nervous excitation of adrenine secretion causes an unfailing hyperglycemia. In this way, emotional states can produce sufficient hyperglycemia to cause glycosuria. This transient "emotional glycosuria" has been observed in a considerable proportion of a group of students after an unusually severe examination and in excited spectators at the close of an athletic contest. Adrenal hyperglycemia is due to the power of adrenine to stimulate the change of glycogen to sugar. The glycogen store of the liver of an animal can be greatly depleted by prolonged adrenal stimulation. This effect of adrenine is in marked contrast to the action of the pancreas hormone which appears to favor the building up of glycogen.

Adrenine has a *stimulating effect upon oxidative metabolism*. Injection of small amounts increases metabolism, as measured by the respiratory exchange, about 20 per cent above the basal level for normal human subjects. The respiratory quotient is usually increased thus indicating that carbohydrate utilization is increased. It has not been shown whether this effect is due solely to the specific dynamic action of sugar, liberated from glycogen by adrenine action, or is caused, in part, by some direct effect upon oxidative metabolism.

Injections of adrenine or excitation of adrenal secretion cause an *increase of muscular power* and of resistance to fatigue in cats and dogs. These facts have been established by ergographic measurements on the artificially excited muscles of anaesthetized animals. To some extent, this effect may be an indirect one, due to the increased sugar content of the blood; but a direct effect of adrenine upon skeletal muscles or at least upon the action of the neuromuscular mechanism, has not yet been eliminated as a possibility.

Adrenine causes *more rapid blood clotting*. After injections of adrenine the clotting time of the blood may be reduced, according to Cannon, from about 3 minutes to as little as 1.3 minutes. The chemical mechanism of this effect is not known.

The Significance of Adrenine.—The physiological effects of adrenine clearly indicate its importance, as pointed out by Cannon, in enabling the animal to meet certain emergencies. The fear and rage which accompany life and death struggles and flight, excite adrenal secretion with its

consequent mobilization of a supply of ready fuel in the form of blood sugar for the use of muscles. The activity of the latter is improved. Blood pressure is raised, the heart is steadied in rate and made to beat more powerfully. The entire animal organism is toned up to maximum working capacity. Even the more rapid clotting of the blood is an advantage in preventing hemorrhage from wounds of combat. The adrenal medulla is the "minute man" for the emergencies of the animal.

These functions of adrenine do not necessarily indicate that it is continuously required during quiet activities and rest of the animal. Blood collected from a normal animal, without in any way exciting it, does not contain adrenine in amounts sufficient to be detected by exceedingly delicate methods. Yet the effects of adrenalectomy, especially the fatal effect, seem to indicate a constant need of the body for adrenine. Further investigation will be required to determine whether the indispensability of the adrenals is due to their power of forming adrenine or to some other function, perhaps that of the cortex.

Adrenine has come to have a considerable use in practical surgery. Locally applied, it serves as a styptic. It constricts small arteries of mucous membranes or of the skin and thus checks the loss of blood from minor operations. Intravenously or subcutaneously injected, adrenine produces a general rise of blood pressure in surgical emergencies. Regular dosage with adrenal preparations has been employed in attempts to counteract and cure Addison's disease. Such efforts have not been conspicuously successful, nor can the effects of adrenalectomy be counteracted, experimentally, by sustained injections of adrenine.

The Adrenal Cortex.—Knowledge of the functions of the adrenal cortex is not yet very complete. Considerable evidence is available to show some relationship between the cortex and the reproductive functions. Abnormalities of the cortex have been reported to be associated, in some cases at least, with premature sexual development. Prevention of sexual development by castration is reported to cause hypertrophy of the adrenal cortex in male animals. The chemical composition of the adrenal cortex includes an exceptional abundance of phosphorus-containing lipoids. Their especial physiological significance remains to be investigated. It is even possible that the cortex may be the indispensable part of the adrenal gland. The fatal effects of adrenalectomy have not yet been proved to be due to the lack of the medulla alone, but may be, in part at least, the result of removing the cortex.

The Gonads.—The reproductive glands, testis and ovary show the most obvious effects of their internal secretion in control of secondary sex characters. The obvious effect of castration is a general failure of development of secondary sex characters, such as hair growth, voice development, anatomical proportions, etc., in human beings, horn growth in stags and goats, development of plumage and growth of combs in birds.

A less familiar instance is that of the clasping muscle of the male frog. It corresponds to the thumb muscle of a human hand. It shows a remarkable hypertrophy at each breeding season but this increased size does not occur if the animal has been castrated. Steinach was able to show that repeated injections of testicular extracts into castrated frogs restored this muscular development. Not only the secondary sexual characteristics are dependent for their development upon the presence of gonads but the external genital organs themselves are dependent for full development upon gonads. Sex behavior and all sex instincts are likewise dependent upon the gonads. Steinach's famous experiments on rats demonstrated the complete reversal of sex instincts after transplanting an ovary into a castrated male or a testis into a castrated female. The male rat, for example, lost all tendencies to fight and acquired the instinct to mate with a normal male and to protect new born rats and even to attempt to suckle them. This work has been confirmed by other investigators, especially by Moore, and has been shown for other species, especially guinea pigs. The sex behavior of human beings is also determined by the presence of gonads. Many psychic attributes of both sexes of all animals may be regarded as secondary sex characters developed under the influence of hormones from the gonads.

Effects of the Gonads on Metabolism.—The tendency of all animals and of human beings to become obese after castration indicates that the gonads favor oxidative metabolism. More definite proof of this is available. Feeding ovarian preparations brings up the rate of oxygen utilization in women by a measurable degree especially after ovariectomy. The effect of ovarian material is, however, much less pronounced than that of the thyroid. Indeed, the gonads may affect oxidative metabolism only indirectly through their influence upon the thyroid.

Metabolism in muscles is probably influenced by an internal secretion of the testis. This is indicated by the results of experiments made by Zoth and Pregl. They took ergographic records of muscular strength and resistance to fatigue in themselves before and after a series of injections of testicular extracts. After this treatment they observed a small but distinct increase of muscular strength and of resistance to fatigue.

Certain phases of metabolism connected with the periodic sex instincts of the female are determined by the ovary. Ovariectomy causes premature menopause in women but this and the distressing symptoms which accompany it can be prevented if, at the time the ovaries are removed, a selected piece of healthy ovarian tissue is grafted into the body cavity. Amenorrhea and dysmenorrhea have been changed to normal menstruation by ovarian graft. The condition of oestrus or "heat" is the periodic activity in the females of many animal species. Its regular occurrence is controlled by the ovary just as menstruation is controlled.

The metabolism of growth is distinctly influenced by the gonads. This is shown by observations upon the effects of castration at different ages. The gonads cause a distinct increase in the rate of growth just before and during puberty but check growth during later years. There is, in general, a longer growth of bones after castration. Castration causes an enlargement of the thymus in young animals and a delay of its involution in mature animals. Castration has been observed to be associated with a tendency to increased size of the pituitary, at least in males.

Sources of Internal Secretions of Gonads.—In the testis, the cells which produce spermatozoa are probably not concerned with the production of an internal secretion. This is indicated by the fact that the spermatogenic cells completely degenerate in a transplanted testis, although the latter shows all signs of furnishing an internal secretion by affecting secondary sex characters, sex behavior and general metabolism. Further proof is found in the results of vasectomy (ligation or resection of the vas deferens). This also causes complete degeneration of the reproductive structures of the testis, but appears to produce no change whatever in its endocrine activity. In contrast to these structures, the so-called **interstitial cells** (cells of Leydig) which form a considerable proportion of the testis and are interspersed among the spermatogenic structures, do not degenerate. They thus appear to be the source of the testicular hormone. They show marked hypertrophy in cases of premature puberty. Steinach has called them the "puberty gland."

In the ovary, interstitial cells, which closely correspond to those of the testis, also occur. All evidence points to the conclusion that they, too, furnish an internal secretion and that it determines certain sex characters of the female. Some of the endocrine activities of the ovary are due to the **corpus luteum**. A corpus luteum forms at the surface of the ovary during and after the extrusion of each ovum and consists of a group of cells of a yellow color, hence the name corpus luteum or yellow body. Feeding or injection of preparations made from corpus luteal material is said to affect conditions of menstruation. Indeed the control of the menstrual and oestrus cycles has been supposed, by some writers, to be completely under control of a hormone produced by the corpus luteum; but other ovarian structures have not been excluded. The corpus luteum appears to influence the development of the mammary gland during pregnancy.

No definite information concerning the chemical composition of any hypothetical hormones from the gonads has been obtained.

The Pineal Gland.—The pineal is a small gland near the base of the brain. It is in contact with the roof of the third ventricle from which it develops embryologically. In human beings it continues to develop until about the seventh year and then begins a process of involution with a

gradual disappearance of its glandular character and a transformation into a fibrous structure. This change becomes complete some time after puberty. Several observers, especially Virchow, have reported that tumorous growths on or near the pineal gland are associated, in children, with the premature development of sex, precocious mentality and an acceleration of growth at ages of from 7 to 12 years instead of at the normal time of puberty. Horrax reported that removal of the pineal gland from young guinea pigs caused premature sex development in both males and females. From these various observations one might infer that the pineal gland furnishes some internal secretion which checks growth and especially checks sexual development; but McCord found that feeding pineal glands to young guinea pigs hastened the development of their sexual maturity. Further investigation is necessary before conclusions as to the rôle of the pineal gland can be drawn.

Other Endocrine Structures.—In addition to the several ductless glands, there are other animal structures which appear to have endocrine functions. The unidentified cells, which are located in the pyloric portion of the gastric mucosa, and which produce gastric secretin were discussed above. Structures of similar function producing pancreatic secretin in the lining of the duodenum were also discussed. Both of these are typically endocrine in their function.

Numerous claims for the *placenta* as an endocrine structure have been put forward but none of them are well established. Some cases of the successful use of placental feeding to check the pernicious vomiting of pregnancy have been reported.

The *prostate gland*, located upon the male urethra near the seminal vesicles close to the bladder, has been investigated as to its possible endocrine activity. It is an externally secreting gland, producing a liquid which serves to dilute the seminal fluid; but this would not necessarily exclude the possibility of production of a hormone. Macht reported that prostate feeding hastens the metamorphosis of tadpoles but less effectively than does the feeding of thyroid.

The *spleen* is a ductless gland and might therefore be regarded as probably endocrine in function. But all attempts to show any indispensable or even important functions for the spleen have been unsuccessful. Its complete extirpation from cats, dogs, rabbits and other species is followed by no significant results. In some species, the hematopoietic function of the spleen is important; but in mammals, it is only temporary and presumably not necessary. The spleen has been shown to be concerned with the production of some of the uric acid formed in the body, but as this substance is a waste product it cannot be called a hormone. On the whole, then, the spleen appears to be a ductless gland without an endocrine function.

Interrelations of the Endocrines.—Certain of the endocrines act in such a way as to reinforce each other and to some extent are able mutually to

substitute for one another. This is true of the thyroid and pituitary, for both of them exert stimulating effects upon growth, development and general metabolism. In a sense, the interstitial cells of the gonads could be grouped with the pituitary and thyroid as stimulating growth, general metabolism and certain phases of development. But each one of these is influenced by the activities of the others and by the activities of other endocrinies. On the other hand, certain of the endocrinies act in a directly antagonistic manner. The pancreatic hormone is opposed, in its action upon carbohydrate metabolism, to adrenine. The former favors glycogen synthesis, the latter its hydrolysis. In view of such relationships as these, there has been a tendency shown in the medical literature of endocrinology to group the endocrinies in two or three sets that are supposed to be mutually reinforcing or antagonistic to one another. It has been supposed that these several groups have their normal rates of secretion so adjusted as to preserve in the healthy body what is called the "endocrine balance." Although this idea is correct so far as it implies that each ductless gland has an optimum rate of secretory activity, which should vary only in accordance with special requirements of the body, yet this idea is very misleading insofar as it implies that most of the endocrinies are actually balanced against one another to cause excitations and inhibitions of secretion. A study of the endocrinies brings out a fact of general and fundamental physiological significance: *An animal is a complex, delicately adjusted machine, operating as a mechanical unit, and any disfunction of any part affects the operation of other parts and thus alters the behavior of the organism as a whole.*

It is true, however, that in many cases a direct influence of one endocrine upon another is detectable. Some of the more important of these effects are the following:

1. The internal secretion of the anterior lobe of the pituitary affects the development of the gonads.
2. Gonadectomy (castration) causes a tendency to increase in the weight of the pituitary.
3. Tumors of the adrenal cortex are associated with premature development or over development of certain of the genital organs.
4. The thymus disappears during or soon after the gonadal development at puberty.
5. Gonadectomy greatly delays the disappearance of the thymus.
6. Abnormalities in the behavior of the thyroid causes changes in the anterior lobe of the pituitary. The latter can replace thyroid in causing metamorphosis of frog larvae.
7. The feeding of corpus luteum substance is said to produce beneficial results in cases of exophthalmic goiter, thus indicating an inhibitory effect of corpus luetum upon the thyroid.

The need of more definite physiological facts is very apparent in endocrinology. This is particularly true because of the confusion that arises from the occurrence of secondary effects due to the action of one endocrine upon another. This confusion is particularly apparent in the medical diagnosis of endocrine disturbances. As such diagnosis and the corresponding organotherapy or surgical treatment have acquired prominence in practical medicine, all studies of endocrinology are especially important.

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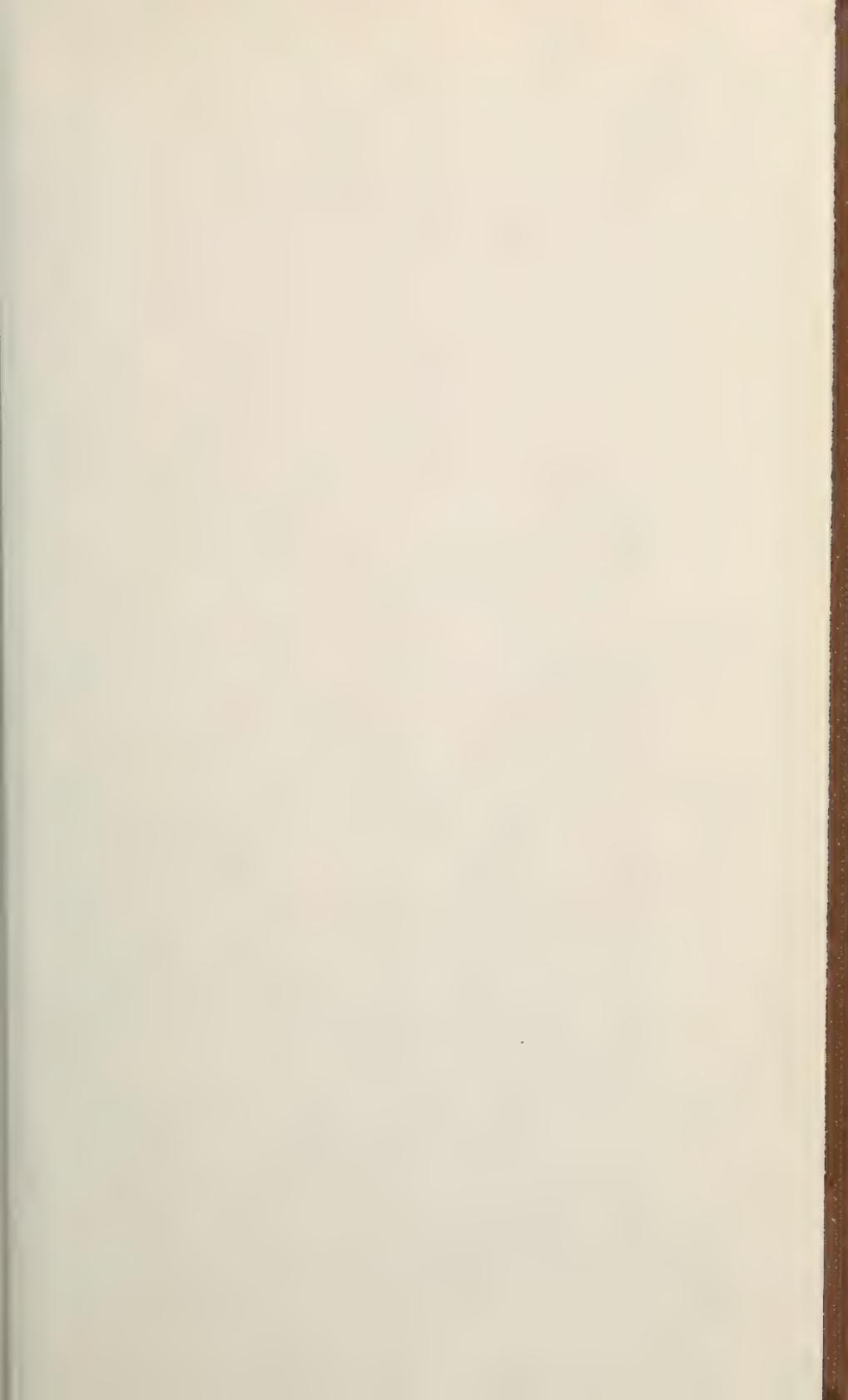
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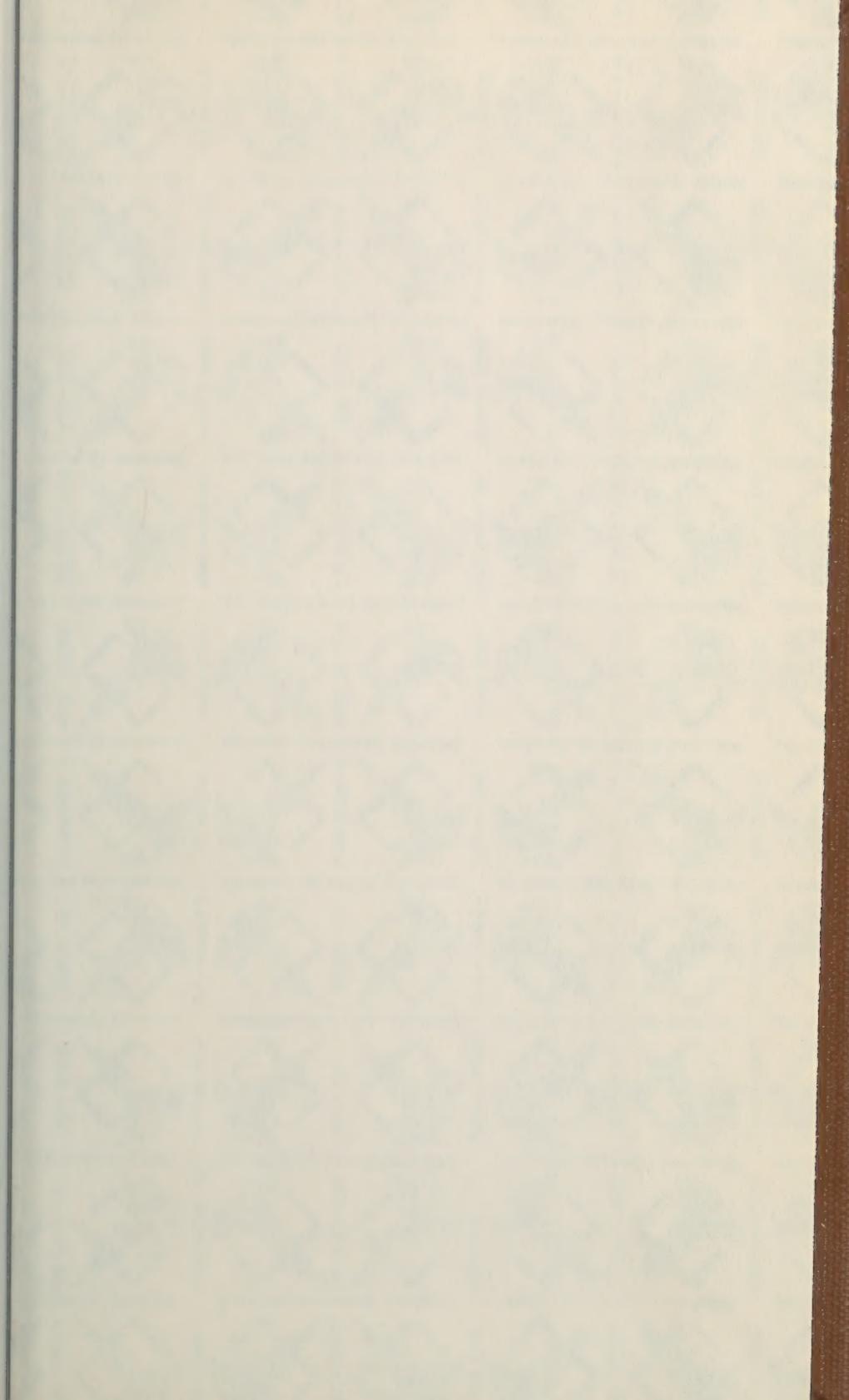
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